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of Pinus contorta Dougl.
var. latifolia Engelm.

UNIVERSITY...of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED...Ph.D.

YEAR THIS DEGREE GRANTED...1974

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THE UNIVERSITY OF ALBERTA

THE MICROBIAL ASSOCIATIONS OF *PINUS CONTORTA* DOUGL. VAR. *LATIFOLIA* ENGELM.

by

© JAMES A. DANGERFIELD

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MICROBIOLOGY

EDMONTON, ALBERTA
SPRING, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Microbial Associations of *Pinus contorta* Dougl. var. *latifolia* Englm." submitted by James A. Dangerfield in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The microflora associated with root systems of young and mature lodgepole pine was investigated by sampling forest grown trees. Counts were performed and expressed on a surface area basis to give a more realistic measure of organism density within the various regions. On this basis, densities increased by an order of 10^5 to 10^6 fold within the rhizoplane with the greatest increases being associated with the smaller feeding roots.

Characterization of rhizoplane and control soil isolates from both young and mature tree root systems demonstrated differences from that reported for agricultural crops. Proteolytic and amylolytic organisms were proportionately reduced within the rhizoplane as were the ammonifiers. The rhizoplane organisms also grew more slowly than the control soil isolates although they responded in greater numbers to the addition of an amino acid supplement of the growth media. The rhizoplane organisms also demonstrated an increased ability to bring about phosphate solubilization.

The chitinolytic organisms were suppressed within the rhizoplane of the mature tree but stimulated by the young trees. With this exception, the rhizosphere microflora of older and younger trees are very similar.

Growth studies in the laboratory, using a sterile plant growth assembly which allows growth at operational rates, indicated that different isolates or groups of organisms can have a significant influence on plant growth and the distribution of this growth.

ACKNOWLEDGEMENTS

The author extends sincere thanks to Dr. F. D. Cook, Professor of Soil Microbiology, Dr. D.W.S. Westlake, Professor of Microbiology and Dr. P. R. Gorham, Professor of Botany for the guidance, encouragement, assistance and patience provided in the supervision and review of this thesis. Special thanks are extended to Mr. F. B. Armitage, Canadian Forestry Service for his moral support and patience while this study was being completed in Victoria, B. C.

Thanks are also extended to Mr. D. Caldwell and Mrs. P. Olsen for able technical assistance provided during the course of this study. Also providing assistance were: Mrs. M. MacGregor-Greer, Misses S. Michaux, S. Nugent, M. Mitchell and P. Stevens and Messrs. M. Cook, D. Gould, A. Jobson, J. D. Smith, J. Wiens, E. Chatelle, A. Craigmyle and A. MacEwan.

The author also thanks the Canadian Forestry Service for providing the opportunity and support for attending graduate school.

The author would like to express his deep appreciation to his family for their encouragement and sacrifices during the course of this study.

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INTRODUCTION

Container-grown seedlings are used in Alberta in large numbers as an economically sound reforestation technique. There are, however, many instances where, in spite of excellent survival, growth is poor. One possible explanation for this is that seedling quality is not appropriate (i.e., too small). What may be required is a plant of defined minimum characteristics (total weight, root collar diameter, top/root ratio, etc.). It has been recently suggested that as management expertise improves, the seedling characteristics will be modified for specific sites. The attempt to maximize growth rates in the initial period and to produce plants of defined characteristics requires a better understanding of all interacting factors. One such interacting factor is the plant associated microflora.

Results collected for a variety of plants and from many areas suggest that micro-organisms may effectively increase plant growth even under ideal nutrient conditions (Miller and Chau, 1970); that the effectiveness of given organisms will vary (Lamb and Richards, 1971); that drought resistance on outplanting may be improved, and that growth stimulation may occur for extended periods after outplanting (Theodorou and Bowen, 1970). Thus it is possible that inoculation of plants, with specific micro-organisms and growth initially under controlled conditions, as occurs with container stock, will result in the production or a larger and more vigorous plant with increased potential for successful establishment and growth on outplanting. To fully evaluate the potential of such a technique, it is desirable that composition, distribution and fluctuation of the natural rhizosphere microflora be understood. In addition, the effect of rhizosphere isolates on the growth capacity of seedlings must be determined.

Because the problems and potentials of such an inoculation technique were recognized, a study of the rhizosphere microflora of lodgepole pine was initiated. This species was selected because of its dominance within the managed forests of Alberta. This tree also has a potentially shorter rotation age than other trees commonly found in the managed forest areas. The objectives of this study were:

a) Characterize and compare the microbial populations of young and mature trees to develop an understanding of the variations in the rhizosphere with age of root and distribution in the soil profile.

b) Determine the influence that rhizosphere isolates have on nutrient uptake and growth capability of pine seedlings.

As part of any research program, a large quantity of literature must be reviewed. Because of the number of interacting factors and the variety of methodology employed, the literature dealing with the rhizosphere phenomenon is extremely confusing and frequently contradictory. For this reason an extensive literature review is included with this report. In addition to emphasizing the tree-related work, it attempts to break the reported results down by important factors, thus clarifying some of the contradictions.

II. Literature Review

The term "rhizosphere" is used to describe that portion of the soil which is subject to plant root influence. This region is a continuum with a demonstrated maximum influence at the root surface, decreasing to no influence at some point in the soil matrix. In areas of very dense root development, as under a cereal grain crop, the whole of the soil may be subject to root influence.

The primary root influence results from an increase in energy supply available to the soil microflora. This is provided either as root exudate or root slough. Other chemical and physical changes occur in the soil as a result of root presence and activity. The interaction of these factors produces the observed rhizosphere effect. Any plant genetic or environmental change such as moisture, light, temperature and nutrition will have an effect on the noted rhizosphere effect.

Generally, the rhizosphere contains a more active microflora. Organisms are present in larger numbers and, in addition, appear to be metabiolically more active. Logically, the proportion of various morphological, physiological and biochemical groupings is changed from that of the non-root influenced soil.

Within the rhizosphere, plant root: micro-organism interactions are most intense. Thus, the potential for microbial influence on plant growth and metabolism is greatest in this region. Various people have demonstrated a positive microbial effect on nutrient uptake, plant metabolism and growth. Unfortunately there are a greater number of reports of failure of the microflora to produce a positive response.

1. Terminology

The term "rhizosphere" was introduced by Hiltner in 1904 to designate that region of soil which is under immediate influence of plant roots (Zagallo & Bollen, 1962). This region has subsequently been redefined and subdivided several times. Perotti (Timonin 1964) used the terms "endophospere" - namely, rhizosphere as defined by Hiltner - and "histosphere" for root surface. Graffs (Timonin 1964) used "outer rhizosphere" and "closer rhizosphere". Clark (1949) suggested the term "rhizoplane", which included external plant root surface together with any closely adhering particles of soil.

It should be recognized that the zone of root influence is a continuum, with root influence gradually decreasing from a maximum at the root surface to no influence at some point in the soil matrix. Logically, no adequate definition of rhizosphere subregions can be made without the appropriate methodology inclusions. Definitions prepared by Louw and Webley (1959), described in the methods section of this report, and Timonin (1964) include precise methodology statements and should serve as standards.

2. Sphere of root influence

Determination of sphere of root influence is dependent upon knowing the length of roots within a defined soil volume and the average radius of root-influenced soil around each root. While the latter is affected by such obvious variables as quantity of material exuded by the root system, soil texture and soil moisture, some general examples will provide guidelines.

Rovira (1953) stated that, under his conditions, the rhizosphere effect was not evident more than 5 mm from the root. Papavizas and Davey (1961) investigated this phenomenon using a multiple core sampler that removed samples at 3 mm intervals outward from the root surface. They found a very pronounced effect in the 0-3 mm sample and a demonstrable effect in the 15-18 mm region. From these results, it is evident that the rhizosphere region is a continuum. The data also suggest that the maximum radius of soil influenced is about 20 mm.

Pavlychenko and Harrington (1934) showed that within a cultivated soil matrix to a depth of 27 inches, any given cubic inch (16.4 cc) of soil contains 5.2 inches (13.2 cm) of root length.

Combining root length data with radius of root-influenced soil permits a rough calculation of volume of root-influenced soil (Table 1).

Table 1

Hypothetical Calculation of Volume of Root-Influenced Soil

Soil volume 1 cubic inch	16.4 cc
Root length (ℓ) 5.2 inch	13.2 cm
Radius of Influence (r2)	1.0 cm
Volume of Influence r	41.4 cc

In the above example, all of the soil volume would be subjected to root influence. Support for the possibility of the complete soil volume being root-influenced is provided by Gyllenberg (1957). He demonstrated, with oats, that the soil flora was initially quite different from that of the rhizosphere and that it changed throughout the season and became similar to the rhizosphere population. Since the changes proceed from the surface to lower horizons, datum on these suggests the change is the result of the whole mass becoming root-influenced.

The only report estimating rhizoplane volume was given by Riley and Barber (1969). In an experiment where 5 soybean plants were grown in pots for 2 and 3 weeks, they found the rhizoplane soil volume to be equal to 0.4% and 0.8%, respectively, of the total soil volume. These results were developed from rhizoplane soil weight data.

3. Soil chemical changes within the rhizosphere

Riley and Barber (1969), working with soybean, demonstrated that bicarbonate ion concentration increased as much as 35 meq per 100 gm in the rhizosphere soil and that pH became more alkaline by as much as 1 unit. The pH change was shown to vary with nitrate ion concentration. Since nitrate is the most common available nitrogen form in agricultural soils, these results suggest the pH of rhizosphere soil in the field would be higher than the nonrhizosphere soil. Riley and Barber (1971) further showed that replacing nitrate-nitrogen with ammoniacal-nitrogen produced a more acidic rhizosphere soil. The magnitude of the differences between nitrate and ammonium-induced pH changes varied with the pH of the original soil. The more acid the original pH, the greater the difference.

In addition to the pH changes, Riley and Barber (1970) noted that the degree of salt accumulation increased with salt concentration of soil and transpiration rate. The maximum net accumulation at 2 weeks was 5 to 15 times the salt concentration of nonrhizosphere soil. Barber and Ozanne (1970), using autoradiographic techniques, also noted the accumulation of salts around root surfaces. They reported that the kind of crop and its specific requirements were determinants in the quantity and quality of salt accumulation.

A major chemical change associated with root development occurs in the accumulation of organic matter. Harmsen and Jager (1963) found from 120-1000 ppm carbon in the soil immediately adjacent to the roots; 120-800 ppm in the surrounding 1-centimeter-thick layer and 0-40 ppm in the more distant remaining soil.

Shamoot _et_ _al_. (1968) used plants grown in a $^{14}CO_2$ environment from the seedling stage to near maturity. Tops and roots were harvested before determination of the residual label in the soil. The magnitude of the "rhizo-deposition" was directly related to the extent of root growth and ranged from 25-49 grams of residual organic debris for each 100 grams of harvested root. These results suggest that a maximum of 486 lbs of organic matter is deposited per acre furrow slice (544.7 Kg/hectare).

4. Plant root exudates

The importance of plant root exudates as the main factor in the establishment of the rhizosphere effect can be inferred from a variety of reported results. Clark (1939), Rouatt and Lochhead (1955), and Papavizas and Davey (1961) noted that the magnitude of the rhizosphere effect was less pronounced when various plant residues were added to the soil. The masking of the rhizosphere effect was also noted with yellow birch seedlings growing in the forest litter horizons (Ivarson and Katznelson, 1960). In addition, Rovira (1956d) was able to establish a rhizosphere soil by watering soil free of plant roots with the exudates of peas and oats.

A study by Parkinson and Pearson (1965), comparing frequency of fungal development on dead and live roots and nylon thread, provides the needed critical data. These authors concluded that stimulation of soil micro-organisms by live and dead roots was mainly due to nutritive materials supplied to the soil. With the nylon thread, there was little evidence of stimulation. Flora supported by dead and living roots differed. These results attest to the importance of plant root exudates as the controlling factor in microbial stimulation within the rhizosphere.

Pearson and Parkinson (1961) and Schroth and Snyder (1961) determined the region of amino acid exudation. Working independently, these authors grew sterile bean seedlings from pre-emergence on filter paper. After tracing the seedling root pattern, the seedling was removed and the filter paper developed for the presence of ninhydrin positive compounds. They both reported that the most active amino acid exuding zone of the root was just behind the tip. No similar experiments designed to determine the exudation zone of other compounds have been reported. It is not illogical to suspect that the tip region may also be the major exudation zone for many other compounds.

A. Quantity and quality of root exudates

Katznelson, Rouatt and Payne (1955) reported that a wide variety of amino acids were liberated by plants. Rovira (1956b) studied the root exudates of peas and oats at two ages. He found quantitative and qualitative differences in the 27 amino acid and related compounds detected. Results from the carbohydrate analysis indicated glucose and fructose were released only during the first 10 days.

Rovira and Harris (1961) examined several plants for vitamin exudation. They found biotin was the only vitamin released in appreciable quantities (9.1-16 nanograms/ml concentrated to 4 plants per ml). Pantothenate and niacin were also present, along with traces of thamine and riboflavin.

Rovira (1962) prepared a comprehensive review of literature containing information on the exudation products (amino acids, vitamins, organic acids, sugars, nucleotides, flavonones and enzymes) of plants. This covered a wide range of conditions, techniques and plant types. For specific details of the literature prior to 1962, readers are referred to this article.

Slankis et al. (1964), in a study with white pine seedlings grown aseptically for 9 months, examined the root exudates following an 8-day exposure to $^{14}CO_2$. They found 9.825% of the supplied activity was exuded and that this activity was incorporated in 35 different compounds. These authors were able to identify only 10 of the compounds. The list of identified compounds included malonic acid, present in the highest concentration, and five other organic acids, oxalic acid, malic acid, glycolic acid and cisaconitic acid. In addition, two sugars, glucose and arabinose, and two amides, glutamine and asparagine, were identified.

Similar experiments with a variety of legume, cereal and vegetable crops have been conducted by Vancura (1964), Vancura and Hovadik (1965) and Boulter et al. (1966). All of them report the relative proportion of various amino acids, sugars and organic acids present in the exudate of plants grown aseptically in liquid or sand culture.

Miller and Schmidt (1965) investigated the root exudates of beans aseptically grown for 24 days in soil. They found that the 1.0 to 2.4 mgm of amino acids exuded per plant was distributed between 8 to 10 amino acids.

Ivarson and Sowden (1969) went still further and examined the amino acid composition of field grown grass rhizosphere soil. They reported that the amino acid concentration was greater in the rhizosphere soil and that the ratio of amino acids from root extract and rhizosphere was different. The variety of amino acids extracted in this study was similar to that reported by Miller and Schmidt (1965) and Rovira (1956b), with aspartic acid, threonine, serine, glutamic acid, glycine and alanine being the more prominent. Spakhov and Spakhova (1970) also examined field rhizosphere, but were interested in a variety of 40-year-old trees. The major limitation of their data is the assumption that all soil in the zone of major feeding root development is rhizosphere soil. As a result, no control soil data are available. In the rhizosphere of pine, they detected the presence of the carbohydrates sucrose and xylose and the amino acids valine, glutamic acid, arginine and cystine plus cysteine. The quantity of these varied independently and seasonally. Also present were oxalic and tartaric acids.

B. Major factors influencing root exudates

a. Light: Rovira (1959) studied the effect of different light intensities on the amino acid exudates of aseptically grown tomato and subterranean clover. In general, greater exudation occurred at higher light intensities, although specific qualitative differences were noted. With clover, serine, glutamic acid and alanine concentrations were greatly reduced with decreasing light intensity. Serine and alanine concentration in tomato exudates increased with decreasing light, while aspartic acid, glutamine, phenylalanine and leucine decreased.

b. Moisture: Katznelson et al. (1955), in a study of several plant varieties (wheat, barley, tomato, pea and soybean), noted that desiccation followed by remoistening produced a substantial increase in the amount of material exuded by the plant root.

c. Temperature: Rovira (1959) studied the root exudates of oats and tomato grown under different temperature conditions. He found that the amount of exudate per unit root weight, in particular glutamic acid and asparagine, increased markedly with temperature. In addition to the quantitative changes, the balance of amino acids was aldered. It should be noted that total plant yield also decreased under the conditions for the increased root exudation. Husain and McKeen (1962), in a study of strawberry root exudates, noted that glycine, threonine, alanine, serine and tyrosine present in 5° and 10° C exudates, were absent in 20° and 30° C exudates. In addition, total quantity of amino acid exuded decreased with increasing temperature. Examination of the carbohydrate exudates indicated glucose present at 10° and 20° C, but not at 30° C. The opposite effect was noted with galactose.

Vancura (1967), in addition to noting that amino acid and sugar exudation by cucumber and maize increased in proportion to temperature, noted that subjecting plants growing at a favourable temperature to 3 days of lower temperature increased exudation and produced qualitative changes in the exudate.

The previously mentioned studies have dealt with aseptically grown plants. Ivarson, Sowden and Mack (1970) studied amino acid composition of brome grass and oat rhizosphere soil maintained at different temperatures. In fertilized plots, the quantity of amino acid in the brome grass rhizosphere soil was greater at 30°C than at 10°C. The reverse occurred in unfertilized plots. For oat rhizosphere soil, the effect of increased temperature was to increase total amino acid exudation on fertilized and unfertilized plots. The increase was greater on unfertilized than fertilized plots where, at some growth stages, the amino acid exudation at 10°C was greater than at 30°C.

From the aforementioned studies, it may be concluded that qualitative and quantitative changes may be expected with different growth temperatures. The magnitude and direction of change is, however, not predictable as it is influenced by the kind of plant and the nutrition of the plant.

d. Nutrition: In an original study, Rovira (1959) was unable to detect consistent effects of changes in calcium nutrition on the exudates from clover, tomato and phalaris grass. Agnihotri (1964) found that foliar urea application produced an increase in the amino acid composition of the plant. This could significantly influence the quantity and quality of plant root exudates.

Bowen (1969) studied the effect of nutrition on amide and amino acid exudation of Pinus radiata. He noted large differences in the different nutritional solutions, with most marked effect occurring in the 2-4 week exudates. The greatest loss occurred in the phosphorus deficient plant, while the least loss occurred in the nitrogen-deficient plant.

In a study of field grown oat and brome grass rhizosphere soil, Ivarson, Sowden and Mack (1970) noted that an increase in soil fertility led to a large increase in amino acid concentration in the rhizosphere soil. For brome grass, this was primarily due to increased aspartic acid, asparagine, glutamic acid and valine exudation.

All of these results suggest that increased soil fertility, which generally increases plant growth, will lead to increased root exudation.

e. Plant growth stage: Rovira (1962) stated in a review article that debris probably becomes more important as an energy source for microbial stimulation as plant age increases. In the early stages of plant growth however, root exudation is the major factor in establishment of the rhizosphere microflora.

Vancura and Hanzlikova (1972) studied the exudates of a variety of germinating seeds and 12- to 14-day-old seedlings. With the exception of wheat, a greater proportion of total nitrogen content in the seed exudate, compared to seedling exudate, was formed by protein and peptide nitrogen than by nitrogen of free amino acids. In addition to a greater proportion of total nitrogen being present as free amino acids, seedling exudates also contained a greater quantity of reducing compounds.

Vancura and Hovadik (1965), in a study of tomato and red pepper, noted quantitative and qualitative differences between root exudates collected at two growth stages (initial and fruiting). Generally, the exudate concentration was lower at the fruiting stage.

Ivarson, Sowden and Mack (1970) studied amino acid composition of rhizosphere soil collected from fields of oats. They found a strong relationship between amino acid concentration and stage of growth. The high value recorded at the third-leaf stage decreased at the fifth-leaf stage, then increased again at heading. Concentrations ranged from 1-19 mgm per gram of dry root or 0.1-2 mgm amino acids per plant. Again variability in quantity and quality of exudate was the rule rather than the exception.

f. Plant genetics: Vancura and Hovadik (1965), following a study of the exudates of a variety of plants, noted that the less plants are related phylogenetically the greater is the difference in the composition of the exudates. Subba-Rao et al. (1962), studying tomato varieties susceptible and resistant to Verticillium alboatrum, found that a greater quantity of material was released over a fixed time period by susceptible varieties. Unfortunately the quantity released was not consistently correlated with resistance or susceptibility. There was, however, evidence of fairly consistent varietal reactions, and closely related varieties behaved similarly.

Buxton (1962) found qualitative differences in the amino acid exudates of Fusarium-susceptible and resistant banana varieties. Of eighteen amino acids detected, thirteen were common to both varieties. In addition, a greater carbohydrate quantity was detected in the susceptible variety exudate.

From these data, it is evident that genetic differences may have a significant effect on the quality and quantity of plant root exudates.

5. Root influence on the soil flora

The most repeated rhizosphere effect that has been reported is an increase in the viable cell count. Table 2, taken from some work by Katznelson (1959), indicates the magnitude of this effect. Results by Rouatt *et al*. (1960) indicate similar values for soybean and barley.

A. Magnitude of the rhizosphere effect

The bacterial flora appears to be stimulated to the greatest extent with the rhizosphere soil count to control soil count (R:S ratio), being about 20 (Katznelson, 1959). Lesser effects are generally reported for fungi and actinomycetes, but the variability is very large. For his studies with poplars, Shipman (1957)

Table 2

Micro-organisms in rhizosphere of wheat seedlings and in control soil (Katznelson, 1959).

Organisms	Numbers (Avg. of 5) Rhizosphere	Control	Significance of Difference*
Major groups			
Bacteria	1,120,000,000	50,000,000	++ 1%
Actinomycetes	38,000,000	5,000,000	++
Fungi	1,160,000	120,000	++
Protozoa	24,100	9,900	+ 5%
Algae	4,500	2,600	n.s.

* Paired t-test

reported that an increase of 2.3-fold was required to be significant at the 1% level. His R:S values varied from 1 to 19. Ivarson and Katznelson (1960), working with yellow birch, reported greater R:S values in mineral than in organic soil horizons. This agrees with the work of Runov and Zhdannikova (1960), who state that "the poorer the soil the more sharply is the rhizosphere effect expressed." This statement is supported by values reported from aquatic environments [R:S 100 (Coler and Gunner, 1969)] and sand dune environments [R:S 130 (Hassouna and Wareing, 1964)]. Webley et al. (1952) reported R:S values varying from 1500 to 15000 for plants developing in a sand dune environment.

Obviously there are many factors that will affect the magnitude of the rhizosphere effect, such as plant type (Starkey, 1929a; Balicka, 1958), plant genetics (Lochhead and Cook, 1961; Timonin, 1966;), and stage of development (Hornby and Ullstrup, 1967; Louw and Webley, 1959a).

B. Initiation and seasonal fluctuation of the rhizosphere

Rovira (1956a) conducted some of the first experiments designed to determine the rate of rhizosphere development. Seeds were germinated and grown in sterile sand. He was able to show a rapid and selective multiplication of the seed flora during the initial stages of germination, and on the root immediately after emergence. In tomato, young root hairs were free from bacteria, while in oats, even young root hairs supported large numbers of organisms.

By sampling seed, emerging roots and more fully developed roots at 3-day intervals for 17 days, Rouatt (1959) was able to characterize and follow the rhizosphere development of wheat. All effects noted at 3 days were maintained or exagerrated with continued plant growth. In addition, he found growth rates of 3-day isolates were greater than those of control soil isolates. The increased growth rates for rhizosphere isolates suggest at least one mechanism for rhizosphere establishment and bacterial selectivity.

In a study of fungal colonization of bean and cabbage roots, Parkinson *et al*. (1963) reported results similar to those reported for bacteria. In these experiments, while there was no fungal development before day 2, the percent root length colonized increased with age over the 10-day experimental period. This is further evidenced by increased density from root tip to crown.

Starkey (1929b), after studying a variety of plants, reported that the plant effect increases with age of the plant and reaches a maximum at the peak of vegetative growth. Shipman (1957), in a quantitative study of the rhizosphere of yellow poplar, found maximum bacterial and actinomycete stimulation occurred at bud break. Ivarson and Katznelson (1960) reported an increase in the rhizosphere effect, based on total count, of yellow birch from bud break onward.

Parkinson and Thomas (1969) studied the rhizosphere fungi of dwarf bean at different growth stages. Using mycelial length measurements as an indicator of activity, they found length increased with increased vegetative growth and decreased markedly at senescence. Comparison of mycelial length data with soil respirometric data showed that both techniques indicated similar trends.

The examples provided and referred to indicate that the rhizosphere effect is very quickly established. This effect increases to a maximum at the time of maximum vegetative activity. It is worthwhile noting that the pattern of root exudation is not unlike the pattern of rhizosphere activity.

C. Taxonomic and morphological changes

a. Morphological changes in bacterial flora: Starkey (1929b), in a study of the organisms associated with sugar beets, alfalfa, field corn, eggplant, rye and apple trees, reported that the gram-negative rods were stimulated by root presence to a greater extent than any of the other groups studied. Lochhead (1940) re-affirmed this by noting that gram-negative rods were proportionately increased, while gram-positive rods, coccoid rods and spore-forming types were less abundant. Lochhead also noted an increased incidence of chromogenic types and motile forms. Stimulation of gram-negatives has been noted in several other studies [King and Wallace (1956), Rovira (1956a) Vasantharanjan and Bhat (1967)].

Webley _et_ _al_. (1952), studying plant succession on a sand dune, noted that gram-negative rods did not appear to be stimulated by root presence, as occurred with agricultural crops. Instead, organisms having affinities with _Corynebacteria_, _Mycobacteria_ and _Nocardia_ represented more than 50% of the organisms from control soil, rhizosphere soil and root surface.

In a study of Douglas-fir mycorrhizal root rhizosphere, Neal _et_ _al_. (1964) found, with one exception, approximately 45% of rhizosphere isolates to be gram-positive. This compares with a range of 13 to 30% for agricultural cereals. In addition, cocci were isolated only from the rhizosphere.

b. Taxonomic changes in the bacterial flora: Brisbane and Rovira (1961) attempted to apply an affinity grouping (a modification of the Similarity Index of Sneath) to rhizosphere isolates. They found that the isolates formed a spectrum rather than a series of distinct groups of organisms. They felt that one of the main limitations of this approach was in the selection of appropriate tests. In an extension of the same study, Rovira and Brisbane (1968) found that an incorporated ecological test (the ability to establish at a high level on wheat roots) became a primary character in the dichotomous key they developed from the data. The Chi values calculated showed that this ability to establish on roots was significantly associated with many characters. Thus the split, based on an ecological test, results in groups which are homogeneous. Skyring and Quadling (1969) also tried to group rhizosphere and soil isolates using a numerical taxonomic system. They were unable to demonstrate a clear differentiation between these organisms.

Clark and Smith (1949) noted that the proportion of Bacillus increased with age of pea and oat roots and that there was a selectivity within the subgroups of aerobic spore formers. Subgroup I (sporangia not definitely swollen, gram-positive) decreased, while Subgroup II (sporangia swollen by oval spores, gram-variable) increased from control soil. Rouatt and Katznelson (1961) reported that Arthrobacter predominate within the soil, while Pseudomonads predominate within the rhizosphere soil.

Leval and Remacle (1968) noted an increase over control soil in the proportion of Arthrobacter and Achromobacter and a decrease in the proportion of Flavobacterium and Pseudomonads within the rhizosphere of Sesleria caerula. These authors (Leval and Remacles, 1969) also reported, for poplar rhizosphere, a decrease in the proportion of Pseudomonads with increasing plant age.

Oswald and Ferchau (1968) studied bacteria associated with mycorrhizal and non-mycorrhizal roots of coniferous species. Members of the Bacillus, Pseudomonas and Micrococcus genus predominated. Of the 51 species identified, 22 were associated only with mycorrhizal roots and 7 only with non-mycorrhizal roots.

As with other root-induced changes, it is apparent that the taxonomic changes reported within the rhizosphere are reasonably specific and influenced by several variables.

c. Taxonomic changes in the fungal flora: Chesters and Parkinson (1959) compared the fungal rhizosphere flora of oats with that of the control soil (Table 3). Examination of this table indicates that there are specific changes in the microflora that are root-induced. Similar root-induced changes have been reported by

Table 3

Major groups of fungi isolated from the rhizosphere during the life of oat plants. (Results expressed as % of the total number of isolates at each isolation time) (Chesters & Parkinson, 1959).

	STAGE					
GROUPS	Young		Mature		Senescent	
	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere	Soil
Mucoraceae	25.0	40.0	24.4	27.0	6.0	33.3
Mortierellaceae	44.4	25.6	26.8	37.8	10.0	25.0
Fusarium	0.0	5.7	4.8	2.7	22.0	5.5
Sterile White mycelium	8.3	0.0	2.4	0.0	0.0	5.3
Sterile dark mycelium	0.0	0.0	2.4	0.0	8.0	2.8
Ascomycetes	0.0	5.7	2.4	0.0	12.0	0.0
Sphaeropsidales	0.0	0.0	9.6	0.0	0.0	0.0
Dematiaceae	0.0	0.0	0.0	0.0	14.0	0.0
Others	22.2	22.8	26.8	27.0	28.0	36.8

other investigators. Peterson (1961) noted that *Phoma* sp. were dominant in wheat root systems, while *Trichoderma* was dominant in soybeans. Parkinson *et al*. (1936) noted that *Fusarium* and *Cylindrocarpon* appeared to become established in the rhizosphere of dwarf beans and barley, while *Mortierella vinocea* and *Penicillium* showed a decrease in incidence with age. Cabbage differed in that *Fusarium* never became a dominant species.

Katznelson *et al*. (1962b) studied the mycorrhizal and non-mycorrhizal roots of birch seedlings. Their data indicates that *Pythium* , *Fusarium* and *Cylindrocarpon* predominate on non-mycorrhizal roots, while *Penicillium* and *Mycelium radicis* predominate on mycorrhizal roots. Kubikova (1963), studying ash root surfaces, reported that *Cylindrocarpon radicicola* formed a predominant component of the surface mycoflora.

From the above-mentioned studies, it is obvious that there are very specific changes in soil mycoflora that are root-induced. It is also obvious, that the change that occurs is in many cases unique to a certain plant type.

D. Changes in individual and population activity

It should be emphasized that most studies concerned with the activity and physiological capability of the microflora have been centered on the bacteria. In several cases, actinomycetes and bacteria are grouped, with little consideration given to separating, the group activities. The lack of physiological characterization of fungi has resulted from the mycologist emphasizing a taxonomic approach. Fungi, because they have a large gene pool, are also recognized to have greater metabolic versatility than bacteria; thus, the reduced interest in their metabolic limitations.

The only paper applying a physiological characterization to fungal rhizosphere isolates (Chatterjee and Nandi, 1967) suggested that legumes stimulated phosphorus solubilizers within the rhizosphere. Addition of insoluble phosphorus also stimulated the fungal phosphorus solubilizers. Phosphorus solubilization is brought about primarily by the action of various organic acids (i.e. 2-Ketogluconic acid).

Abraham and Herr (1964) studied the rhizosphere actinomycete flora of soybean and corn rhizosphere soil and non-root influenced control soil. They found that significantly more of the rhizosphere isolates were capable of starch hydrolysis and that the percentage within soybean was significantly greater than that within corn rhizosphere soil. No differences in ability to hydrolyze cellulose, liquify gelatin or reduce nitrate were noted. Kaunat and Bernard (1969) reported that all of the actinomycete species in the rhizosphere of fifteen cultivated and wild plants were capable of liquifying pectin gel. No similar evaluation of organisms isolated from control soil was made.

Although rather limited, the above-noted references are the only reports found that dealt with fungal or actinomycete rhizosphere soil isolate physiological capabilities. Of necessity, the remainder of section "D" shall deal only with the physiological capabilities of the bacterial flora.

a. Growth rate: Lochhead (1940) reported that the rhizosphere contained a higher percentage of organisms which develop well on nutrient agar. Rovira (1956c) reported that isolates from the rhizosphere generally grew more prolifically (determined by optical density readings) than soil isolates when placed on similar media. The mean response to root exudate by rhizosphere isolates was 1.6 turbidity units and by soil isolates 0.8 turbidity units. Rouatt and Katznelson (1957) reported that the trend is to an increase in number of rapidly growing forms in the rhizosphere of all plants tested. They did, however, note a great deal of variation dependent upon the plant. Rouatt (1959) noted that this difference in growth rate between soil and rhizosphere isolates was evident 3 days after seed germination.

b. Nutritional requirements: The nutritional classification of soil bacteria was introduced by West and Lochhead (1940). In the original work maximum growth was used as an indication of a specific need. A basal medium, containing only glucose and inorganic salts, was amended with various organic constituents (amino acids, vitamins and yeast extract) to produce four media of increasing complexity. Bacteria from the rhizosphere of flax and tobacco were found to possess more complex nutritional requirements than isolates from the corresponding control soils. In this case, the rhizosphere isolates responded to amino acid and growth factor supplements to a greater extent than control soil isolates. Lochhead and Thexton (1947) modified the original media for a study of mangel rhizosphere soil isolates. They reported that the proportion of isolates capable of maximum growth on simple media (glucose and amino acid additives) increased within the rhizosphere, whereas the proportion of groups requiring complex media (yeast and soil extract additives) decreased from the value in the soil.

Timonin and Lochhead (1948), in a study of distribution of micro-organisms within the rhizosphere, noted that organisms requiring amino acidsfor maximum growth were more numerous in root sections further from the base of the stem, probably in the root tip region.

Wallace and Lochhead (1949) studied the rhizosphere microflora of six different agricultural crops at two stages. They also reported a larger proportion of rhizosphere isolates with simple nutritional requirements as compared to soil isolates. Soil isolates contained a greater proportion of organisms with complex nutrient requirements. After a more detailed study with the amino acid-requiring organisms, Wallace and Lochhead (1950) reported that the sulfur-containing amino acid group, and methionine in particular, was required by the majority as a supplement for maximum growth. These amino acids have only been found in very small amounts, if at all, in root exudates of a large number of plants.

Wallace and King (1954) examined the rhizosphere and control soil microflora from field grown oats and barley at two stages. Following statistical analysis of the data, they concluded that root excretions in the rhizosphere had little or no effect on the equilibrium of the nutritional groups. In response to this work, Lochhead and Rouatt (1955) compiled a list of data from all similar experiments carried out at Ottawa to show the consistent preferential stimulation of organisms requiring amino acids for maximum growth within the rhizosphere. Rouatt et al. (1960) went further and carried out a statistical evaluation of data collected in a study of isolates from wheat, barley and soybean rhizosphere. Again, they noted a significant increase in the proportion of bacteria requiring amino acids for optimal growth within the rhizosphere. Skyring and Quadling (1969) reported that rhizosphere isolates were generally less demanding nutritionally than soil isolates.

It would appear that, for agricultural crops, the rhizosphere microflora contains an increased proportion of amino acid-requiring organisms as compared to the non-root-influenced soil.

Several studies have been conducted with forest vegetation. Ivarson and Katznelson (1960) indicated that isolates from yellow birch rhizosphere in a soil horizon containing 17% organic matter were not nutritionally different from control soil isolates. The differences in requirement for simple and complex media, reported by other authors for other crops, were evident in a soil horizon containing less than 2% organic matter. This observation is probably the direct result of the different organic contents. Rouatt and Lochhead (1955) reported that the incorporation of plant material in the soil produced a soil flora similar in nutritional requirement to that of typical rhizosphere flora.

Katznelson et al. (1962b) compared mycorrhizal and non-mycorrhizal root rhizospheres of yellow birch. They noted an increase in the incidence of organisms with complex nutritional requirements on the mycorrhizal roots. The majority of isolates from both roots (64-67%) required amino acids for optimal growth.

Leval and Remacle (1968) studied the rhizosphere flora of a native grass. They also noted the selective stimulation within the rhizosphere of organisms requiring amino acids for optimal growth.

From the preceding review of the literature, the selective stimulation around roots of an amino acid-requiring microflora could almost be considered a universal characteristic.

c. Physiological Capabilities: It is in the literature dealing with the physiological capabilities of the rhizosphere microflora that the greatest confusion exists. Much of this is the result of differing methodology. To provide a proper base for comparison in the section that follows, an indication of the methodology is included.

1) Ammonification. In a study of mangel root flora, Katznelson (1946) noted an effective stimulation of ammonifiers within the rhizosphere of the older plants. He employed a most probable number (MPN) technique for this study. The data (MPN) of Timonin and Lochhead (1948) suggested a proportional increase in ammonifying organisms within the rhizosphere of tobacco with increasing plant age. Katznelson and Rouatt (1957a), in a study of wheat, oat, rye and barley rhizosphere (MPN), demonstrated a stimulation of ammonifiers. The magnitude of the increase was variable. Although no rhizosphere to soil total count ratios were presented, it would appear that, with the exception of oats, the magnitude of stimulation would be greater than that for total counts and thus a proportional

increase in the ammonifying population. Individual isolates from this sampling were also characterized and again, with the exception of oats, the authors demonstrated an increase in the percentage of isolates capable of ammonification. In this study, the data obtained, employing a most probable number technique (MPN) and an individual isolate characterization, are directly comparable.

Rouatt et al. (1960) demonstrated a stimulation of ammonifiers (MPN) within the rhizosphere of wheat which was significant at the 99% level of probability. Using a similar technique, Rouatt (1959) had noted this stimulation on the roots of 3-day-old seedlings. Neal et al. (1970), in a study of the rhizosphere of three wheat varieties, noted a stimulation of the ammonifying population that was significantly different from the control at the 95% level of probability. Each variety was also significantly different from the other varieties.

Studies employing forest species are less common. Ivarson and Katznelson (1960) noted a stimulation of ammonifiers (MPN) within the rhizosphere of yellow birch 7 and 28 weeks after breaking dormancy. Tribunskaya (1955) reported that a large number of ammonifiers were present in the rhizosphere of pine seedlings, but the data were not reported in a manner that allowed a proportional comparison with control soil. Neal et al. (1964), in a characterization study of isolates of Douglas-fir mycorrhizal rhizosphere, reported that the proportion of ammonifiers decreased from that of the control soil. Rambelli (1967) was also unable to detect a proportional increase in the ammonifiers of radiata pine mycorrhizal rhizosphere. Leval and

and Remacle (1969) reported that ammonifiers were present within the rhizosphere of poplar in very large numbers, but did not report soil values for comparison.

From the material reviewed, it would appear that the stimulation of ammonifiers, which occurs in the rhizosphere of agricultural cereal crops, is rarely reported to occur with forest vegetation.

ii) Denitrification and nitrate reduction. Katznelson (1946) reported that denitrifiers were selectively stimulated (MPN) in the rhizosphere of old mangel plants. This was not the case with young plants. Timonin and Lochhead (1948) noted the same phenomenon is tobacco rhizosphere (MPN). King and Wallace (1956) characterized isolates from the rhizosphere of young and mature oats and barley plants and found a significant stimulation in the proportion of nitrate reducers only within the rhizosphere of young oat plants. Katznelson and Rouatt (1957a) reported an increase in the proportion of denitrifiers in the rhizosphere of 5-week-old wheat and mature rye and barley. No increase was noted for the oat rhizosphere (MPN). Other reports on an increase in the number of denitrifiers have been made by Balicka (1958) for vetch and rye and by Rouatt (1959) for spring wheat. Neat *et al.* (1970) noted a stimulation which was significant at the 95% level in 2 of 3 spring wheat varieties studied (MPN).

With forest vegetation, the reports are more variable and more poorly documented. Runov and Zhdannikova (1960) reported a large number of denitrifiers in the rhizosphere of spruce and birch.

Leval and Remacle (1969) made a similar observation with poplar (MPN). Unfortunately, neither of these authors has provided control soil data for comparison.

Kozlova et al. (1936), in contrast, reported very low numbers of denitrifiers within the rhizosphere of birch, spruce and Scots pine. Egorova and Raguotis (1968) found that denitrifiers were present in greater numbers within the rhizosphere of birch than spruce.

In a study of Douglas-fir mycorrhizal and non-mycorrhizal rhizosphere isolates, Neal et al. (1964) noted a general decline in denitrifier proportion from control soil. Rambelli (1967) reported no differences detected in denitrifier proportion between control and radiata pine mycorrhizal rhizosphere.

It would appear that denitrifiers are more commonly stimulated within the rhizosphere of agricultural crops than forest vegetation. This is not altogether unexpected, since nitrate is not the major available nitrogen form in forest ecosystems as it is in agricultural ecosystems (Corke, 1958).

iii) Proteolysis. Lochhead (1940), in a characterization of isolates from control and rhizosphere soil of tobacco, corn and flax, reported a higher incidence of gelatin liquifiers from the rhizosphere. The magnitude of the change varied with plant variety and type. Timonin and Lochhead (1948) noted an increase in the proportion of casein hydrolyzers (MPN) with increasing root age. Balicka (1958) reported an increase in proteolytic flora within the rhizosphere of rye and vetch and Rouatt (1959) reported an increase in gelatin hydrolyzers for wheat rhizosphere.

In contrast to the above, King and Wallace (1956) reported a decrease in the proportion of gelatin hydrolyzers in oat and barley rhizosphere.

Remacle (1963) examined the microflora associated with plant roots found in three soils characterized by mor, moder and acid mull organic surface horizons. He found the plants present in the moder and mull stimulated the proteolytic flora, whereas those in the mor did not. Leval and Remacle (1969) reported that proteolytic organisms were present in the rhizosphere of poplar in large numbers, but comparable soil data were not presented.

The general stimulation in proteolytic flora for a wide variety of plants may simply be a reflection of the increased microbiotic activity associated with root environments.

iv) Nitrification. Katznelson (1946) noted a numerical increase in nitrifiers present within the rhizosphere of mangels. Comparison of rhizosphere count to soil count ratios for nitrifiers with that for total flora indicated that, proportionally, the nitrifiers were decreased. Timonin and Lochhead (1948) presented data from a study of tobacco rhizosphere microflora that suggests that the nitrifiers formed a decreased proportion of the total compared with the control soil microflora. In a study of mulberry, Vasantharajan and Bhat (1968) reported a 2-fold increase in the number of nitrifiers from control to rhizosphere soil. Associated with this was a 13-fold increase in total numbers, which resulted in a decreased proportion of nitrifiers.

Kozlova *et al.* (1963) reported that nitrifiers were nearly absent from the rhizosphere of Scots pine, spruce and birch. This was also true for the surrounding soil.

The only exception to the previously reported negative response of nitrifiers to the rhizosphere is a study by Rambelli (1967). Here radiata pine mycorrhizal rhizosphere isolates contained a higher proportion of nitrifiers than that found in control soil.

v) Nitrogen Fixation. Starkey (1929b) noted that, for the eight plants he studied, there was stimulation of numbers or organisms capable of growth on nitrogen-free mannite agar. This stimulation was equal to or greater than that for the total flora. Katznelson (1946) found a proportional reduction in *Azotobacter* around mangel roots. Leval and Remacle (1969) found that both anaerobic and aerobic nitrogen fixers of poplar rhizosphere represented a very low percentage of the total physiological capability, but did not provide comparable control soil data.

Rambelli (1967) again provides the only reported exception to a decreased proportional nitrogen fixing component within the rhizosphere. The flora association with radiata pine mycorrhiza contained a larger percentage of organisms capable of growth on nitrogen-free media.

vi) Methylene Blue Reduction. Katznelson and Rouatt (1957a) introduced the reduction of methylene blue as a characterization of the rhizosphere microflora. Hopefully this could serve as an indicator of the oxidative capacity or metabolic activity of the soil microflora. They found the number of methylene-blue-reducers increased in the rhizosphere of wheat, oats, barley and rye.

Examination of individual isolates from these various rhizospheres indicated an increase in the percent methylene-blue-reducers only for wheat and barley. Rouatt (1959) demonstrated that the significant stimulation associated with wheat was present in seedlings less than 3 weeks old. He used a most probably number (MPN) technique in this study.

Ivarson and Katznelson (1960) were unable to demonstrate a proportional increase in the number of methylene-blue-reducers (MPN) in yellow birch rhizosphere soil until the fifth and final root sampling, 28 weeks after breaking dormancy. Since these roots were all located within a soil horizon containing a high percentage of organic matter, some masking is to be expected. At 28 weeks, roots that had penetrated the B horizon were sampled. Around these roots, the stimulation was much greater (MPN). A characterization of isolates from the two horizons failed to reproduce the reported selective stimulation of methylene-blue-reducers within the rhizosphere.

Katznelson et al. (1962b) reported a greater number of methylene-blue-reducing organisms associated with mycorrhizal than non-mycorrhizal roots.

vii) Glucose Oxidation. Lochhead (1940) reported a consistently greater number of isolates, from six plants studied, which produced acid from glucose. King and Wallace (1956) reported that isolates from oat and barley rhizosphere did not differ significantly from control soil isolates in this capability.

Katznelson and Rouatt (1957a) reported a stimulation of organisms capable of producing acid from glucose within the rhizosphere of wheat, rye and barley, but not oats. Results were the same whether the characterization was carried out using a most probable number (MPN) technique or a sampling of individual isolates. Rouatt (1959), in a study of wheat less than 3 weeks old, was unable to detect a selective stimulation of these acid producers.

A larger number of organisms capable of acid production (MPN) were found in yellow birch mycorrhizal than in non-mycorrhizal rhizosphere soil (Katznelson et al. 1962b).

Elwan and Diab (1970a, 1970b) noted the lack of stimulation of acid producers within the rhizosphere soil of a variety of desert plants.

viii) Cellulose Hydrolysis. Katznelson (1946) noted an increase in numbers within the rhizosphere of mangels, but this increase was much less than in total number of organisms. Timonin and Lochhead (1948) reported that organisms capable of cellulose hydrolysis were present in very low numbers within the rhizosphere of tobacco.

Rouatt (1959) noted a very large stimulation of these organisms in the rhizosphere soil of wheat seedlings. Rouatt et al. (1960) found, with older wheat, that this stimulation was significant at the 99% probability level. Saric (1967) also noted the stimulatory influence of wheat upon the cellulose hydrolyzers.

Runov and Zhdannikova (1960) reported a stimulation of these organisms within the rhizosphere of spruce and birch. Koslova et al. (1963), in contrast, reported that cellulose hydrolyzers were almost absent from the rhizosphere of birch, spruce and Scots pine. A similarly low activity was reported in the rhizosphere of poplars (Leval and Remacle, 1969).

The cellulose hydrolyzers have been found to be stimulated in the rhizosphere of Sesleria caerulea, a grass (Leval and Remacle, 1968). Elwan and Diab (1970, 1970a) have also reported the stimulation within the rhizosphere of a variety of desert plants. In the latter case, the numerical stimulation is less than that for the total organisms count, indicating the lack of preferential stimulation.

ix) Starch Hydrolysis. King and Wallace (1956) reported that the percentage of organisms capable of starch hydrolysis decreased in the rhizosphere of oats and increased in the rhizosphere of barley. Rouatt (1959), studying the initiation of the rhizosphere effect with wheat, found organisms capable of starch hydrolysis were stimulated more than any other group of organisms he examined. Leval and Remacle (1968) found within grass rhizosphere that the ability to hydrolyze starch was numerically one of the most important characteristics they studied. This was also true for the rhizosphere microflora of poplar (Leval and Remacle, 1969). Unfortunately, in the last two papers mentioned no comparative soil data were presented.

In contrast, Neal et al. (1970) found that the number of starch hydrolyzers in the rhizosphere of wheat increased no more than the total number of organisms.

x) Pectin and Hemicellulose Hydrolysis. Balicka (1958), in a study of rye and vetch rhizosphere, stated that excretion and exfoliation of roots created an environment favorable for the growth of organisms of the carbon cycle. This was especially true of hemicellulose and pectin hydrolyzing bacteria. Leval and Remacle (1969) found that about 1% of the rhizosphere microflora of poplar was capable of hemicellulose and pectin hydrolysis.

xi) Phosphate solubilization. Katznelson and Bose (1959) examined wheat rhizosphere and control soil isolates for the ability to solubilize phosphate. They were unable to demonstrate a selective stimulation of this flora. Louw and Webley (1959) found that the number of dicalcium phosphate solubilizers increased within the rhizosphere of oats, but they were not preferentially stimulated. Sperber (1958) noted both a numerical and proportional increase in phosphate solubilizers within the rhizosphere of clover, ryegrass, perennial ryegrass and wheat.

Katznelson *et al*. (1962a) carried out plate counts to determine the number of phosphate solubilizers in control soil and the rhizosphere and rhizoplane of corn, red clover, flax, oats, barley and yellow birch seedlings. Only in the case of barley was there any indication of selective stimulation. Oats had an inhibitory effect. With the other crops, there were large numerical increases over the control soil, but no proportional change within the population.

In addition to the foregoing detailed observations, various other authors (Chunderova, 1964; Elwan and Diab, 1970a; Louw, 1970; Raghu and MacRae, 1966) have noted the numerical increase of phosphate solubilizers within the rhizosphere, but few have reported a selective stimulation.

E. Biotic processes within the rhizosphere

To this point the literature has suggested that within the rhizosphere there generally exists a microbial population with a more rapid growth rate which is enriched in organisms capable of bringing about various organic transformations. This trend of increased organic transformation is most strongly expressed for the nitrogen-containing materials. Since the ultimate concern is the influence of the organism on the plant production, a prime interest is the significance of the biological processes in rhizosphere soil as it relates to observed microbiological capabilities.

Rovira (1956d) established large volumes of rhizosphere soil by adding pea root exudates to soil for several consecutive days. The resulting degree of microbial stimulation within this artificial rhizosphere was reduced from the true rhizosphere. In the absence of organic matter additions, he was unable to detect differences in availability of nitrogen or phosphorus between control soil and artificial rhizosphere soils. Phosphorus availability also was not influenced by nucleic acid addition to the soil. When peptone was added however, nitrate formation was more rapid within the rhizosphere soil. From a study measuring oxygen uptake, it was apparent that the treatment with root exudate did not influence the normal soil O_2 uptake, but the decomposition of readily available organic substances (*i.e.*, glucose) was more rapid.

Katznelson and Rouatt (1957b) noted greater oxygen uptake by rhizosphere than by control soil. In these studies, rewetted air-dry soils were used. The authors also observed a more rapid response of rhizosphere soil to the addition of readily available organic substrates (*i.e.*, casamino acids).

Guirguis *et al*. (1969a, 1969b) also noted that added amino acids were degraded more rapidly in rhizosphere than in non-rhizosphere soil. They noted that lag phase was shorter and maximum oxygen consumption occurred sooner in rhizosphere than in non-rhizosphere amino acid-supplemented soils. Final oxidation, however, was not different.

The previously mentioned studies suggest increased activity within the supplemented rhizosphere and increased ability to rapidly respond to readily available organic material. Neal *et al*. (1967) presented data to indicate this observation varied with plant type and was influenced by root condition. They found that adding a slurry of Douglas-fir suberized root or red alder mycorrhizal root to non-rhizosphere soil stimulated oxygen uptake. This effect was magnified if glucose was added at the same time. In cases where Douglas-fir mycorrhizal or red alder suberized root slurries were added, oxygen uptake was suppressed.

Kozlov (1964) noted increases in peroxidase, polyphenoloxidase, dehydrogenase and urease activities within the rhizosphere of beet, maize and horse-bean.

The material presented within this section demonstrates that the previously mentioned changes in microbial characteristics associated with the rhizosphere are indicative of changes in biological activity within the rhizosphere soil.

6. Environmental factor influence on the rhizosphere microflora

In the preceding sections, an attempt has been made to provide a summary of the effect of various environmental factors on root exudation and to provide a summary of the characteristics and capabilities of the general rhizosphere microflora. Quite logically, if root exudates are the major control in the establishment of the rhizosphere, factors that affect root exudation must also affect the rhizosphere microflora. This section tries to provide some general observations on this interrelationship.

A. Light

Harley and Waid (1955) noted a distinct change in root surface flora of beech as light intensity was reduced. Associated with the increased sugar with high light intensity was an increase in mycorrhiza formers and a decrease in phytopathogens. Rouatt and Katznelson (1960) noted a reduction in number of bacteria, methylene-blue reducers, ammonifiers and glucose fermenters on roots of plants grown under conditions of low light intensity. These observations are not unexpected in view of the effect of increased light intensity leading to an increased root exudation. In addition, Coler and Gunner (1969) noted that the rhizosphere carrying capacity of a water plant (Duckweed) appears to be a function of photosynthetic capability.

By way of contrast, Peterson (1961) noted that the fungal root colonization pattern of shaded and unshaded wheat and soybean was the same.

B. Moisture

Clark (1947) showed that total microbial content was sharply higher for rhizosphere samples from drier soils. It was suggested that this was due to increased root growth. Peterson, Rouatt and Katznelson (1965) studied wheat rhizosphere and rhizoplane microflora in soils adjusted to 30, 60 and 90% of moisture holding capacity. Bacterial counts, fungal counts and numbers of organisms capable of methylene-blue reduction, glucose fermentation and ammonification all increased as soil moisture decreased. This also is not unexpected in view of increased root exudation under dry conditions.

In addition to the physiological group changes, certain taxonomic changes in population composition occurred as a result of the different moisture conditions. Pseudomonas predominate under low and intermediate moisture, while Arthrobacter, Bacillus and Cytophaga dominate at high moisture. Some fungal groups were restricted by the high soil moisture level (Mortierella, Rhizopus, Chaetomium, Curvularia and Helminthosporium).

These authors noted that there was more root hair formation in the drier soil. Pearson and Parkinson (1961) noted that maximum root exudation occurred within the region of root hair formation. It is thus possible that the increased root hair formation is accompanied by increased root exudation and is a partial explanation for the increased rhizosphere activity.

In a study of dwarf bean, Taylor and Parkinson (1964) noted that Penicillium decreased with increasing moisture, while

Cylindrocarpon increased in the same sequence. They used soil adjusted to 30%, 50% and 70% of moisture holding capacity. In a study of soybean root surface fungi, Ivarson and Mack (1972) reported that soil moisture influenced the frequency of only one genus, Gliocladium. These authors worked over a very narrow moisture range between 70 and 100% of moisture holding capacity.

C. Temperature

Rouatt et al. (1963) carried out a study using wheat and soybean grown under three different temperature conditions. For wheat, the increasing temperature reduced the rhizosphere effect, while for soybean, the opposite effect was observed. The response noted for total number of micro-organisms within the rhizosphere at the different temperatures also held true for the various physiological groups examined; methylene-blue reducers, ammonifiers and glucose fermenters. With increasing temperature, the proportion of gram-negative flora in control and soybean rhizosphere soil increased, while within wheat rhizosphere soil, this proportion decreased. These same authors reported that Fusarium and Cylindrocarpon spp. were the most frequent isolates from soybean at low temperature, while Mucor, Rhizopus, Rhizoctonia and Gliocladium showed a greater incidence at higher temperature. With wheat, non-sporing dark species predominate at high temperature and non-sporing hyaline types at low temperature.

Taylor and Parkinson (1964) conducted a similar study on the fungal flora associated with dwarf bean roots. They noted increased root colonization with increased soil temperature.

Ivarson and Mack (1972), in a study of soybean root mycoflora, demonstrated that changes in soil temperature markedly affected the relative frequency of isolation of 12 fungal genera. In general, Rhizoctonia were more prevalent on roots at higher temperature, whereas Cylindrocarpon appeared to be well-adapted at low temperatures. These results agree with the observations of Rouatt et al. (1963). In Ivarson and Mack's work, the maximum soybean yield occurred at the high soil temperature.

Since the plant root exudate quantity and quality has been shown to change with temperature, the above-mentioned results confirm the expected observation of changes in microbial activity within the rhizosphere as temperature varies. Maximum rhizosphere activity is generally associated with maximum vegetative growth; thus, the maximum rhizosphere effect at higher temperatures for soybean was predictable.

D. Nutrition

In a study of the rhizosphere microflora of mangels, Katznelson (1946) noted that manure-fertilized soil produced plants with a more pronounced rhizosphere effect in the same time period. Absalyamova (1963) demonstrated that fertilization produced a greater rhizosphere effect. This observation is not unexpected as increased soil fertility leads to an increase in root exudation.

Other Russian workers (Tyutyunnikov and Pronin, 1965) also reported that fertilizers increased the number of various physiological groups of micro-organisms in the rhizosphere. In this study, the fertilizer that produced the greatest plant yield also produced the greatest rhizosphere stimulation.

Loutit, Hillas and Spears (1972) found that molybdenum fertilization of radish significantly influenced the composition of the rhizosphere flora. Pseudomonas, Achromobacter, and the Flavobacterium-Eriwinia group were proportionately increased (99.9% level of significance) in Napier soil, while Bacillus was proportionately decreased (99.9% level of significance) in Hasting soil.

Fertilization, as expected, leads to changes in magnitude and quality of the rhizosphere effect. The qualitative change is, however, influenced by several other variables and interaction.

E. Soil

Peterson (1958) noted that soil type influenced the nature of the fungal flora associated with plant roots. He suggested that this observed effect in the different soils tested was due largely to the influence of soil reaction. Parkinson and Clarke (1961), in a study of leek root mycoflora, substantiated the observation made by Peterson (1958). They felt this could be explained by the varying sensitivity of the individual root surface fungi to soil reaction. Taylor and Parkinson (1964), in a study of dwarf bean root mycoflora, noted that Fusarium are characteristic isolates of roots in acid soils, whereas Cylindrocarpon are characteristic isolates of roots in alkaline soil. This is similar to the previously mentioned data of Peterson (1958). Loutit, Hillas and Spears (1972) identified (Skerman's Key) some 1500 rhizosphere isolates of radish grown on two different soils under identical conditions. Taxonomically, the rhizosphere organisms were significantly different. *Pseudomonus* and *Achromobacter* were present in higher proportion in Hasting soil (99.9% level of significance), whereas *Arthrobacter*, *Nocardia* and *Streptomyces* were present more frequently in Napier soil (99.9% level of significance).

It would appear that the plant assumes a secondary influence relative to the primary soil influence in the establishment of the rhizosphere microflora.

7. Micro-organism influence on plant systems

Many studies of the rhizosphere phenomenon and its associated flora have been undertaken to provide basic information. From this it is hoped will come the understanding necessary to successfully manipulate the rhizosphere microflora for the plant's benefit. What are the potential benefits of any manipulation?

A. Influence on nutrient uptake

Gerretsen (1948) provided much of the impetus for these studies by demonstrating that phosphate uptake was increased over sterile controls by inoculation with a mixed flora. This observation held true for a large number of plants grown under phosphorus-deficient conditions supplemented with an unavailable inorganic phosphorus source.

In contrast, Akhromeiko and Shestakova (1958) found that the presence of micro-organisms in oak, ash and maple rhizospheres reduced uptake in the short term and had little or no effect in the long term (2 months). Szember (1960) also found that, for a number of vegetable crops, the addition of organic phosphorus decomposing organisms did not enhance phosphorus uptake. Subba Rao et al. (1961), in some work with a number of tomato varieties, found that organism inoculations reduced phosphorus uptake. They also noted a reduced sulfur uptake in the presence of this Fusarium inoculum.

Rempe and Kaltagova (1958) found that a mixed rhizosphere inoculum increased the nitrogen, phosphorus and potassium content of maize in pot experiments. Bowen and Rovira (1966) also reported that a mixed microbial inoculum increased the short-term phosphorus uptake of tomato, clover and wheat two-fold over uptake by sterile controls.

Barber (1966) and Barber and Lougham (1967) demonstrated that some of the variability in observed results to date may be accounted for by differences in phosphorus nutrition. They found, with barley, that at low phosphorus levels (0.001 ppm), the microorganisms' presence reduced uptake, while at high phosphorus levels (10 ppm), the organisms have no effect. Barber et al. (1968) found that the majority of the phosphorus uptake at low concentrations was located at the root surface, thus suggesting fixation within the microbial tissue. A similar localization of phosphorus in maize roots was noted by Crosset (1967). Presumably, under deficient conditions, the organisms are effective competitors for any available phosphorus.

Barber (1969) further demonstrated the importance of direct nutrient condition comparisons with some studies of nitrogen assimilation. He found that a mixed soil inoculum increased nitrate-nitrogen uptake two-fold over the sterile control. This same inoculum, however, reduced ammoniacal-nitrogen uptake by one third.

The material quoted to this point would suggest the absolute need for complete knowledge of the nutritional conditions of any experiments before proper comparisons of organism influence can be made. In addition, it appears that a mixed microbial culture will more frequently produce a positive response than will a single organisms inoculum.

Fisher and Stone (1969) have provided some indirect evidence of the importance of the microflora to enhanced nutrient uptake. In abandoned agricultural land that has been reforested with pine, herbaceous vegetation has been found to grow larger and have a higher nitrogen and phosphorus content than adjacent vegetation on the same non-reforested soil. The reforested soil contains a significantly higher amount of available nitrate-nitrogen, ammoniacal-nitrogen and phosphorus, but the sites were not significantly different in total nitrogen or phosphorus. They suggest the conifer selects a microbial flora that mineralizes some portion of organic material that was resistant to mineralization under the previous vegetation.

Miller and Chau (1970) grew soybean plants in a completely sterile system in soil which indicated an adequate nutrient pool. While there was no direct microbial influence on nitrogen, phosphorus and potassium, the micro-organism presence significantly increased calcium, magnesium, iron, aluminum and molybdenum levels.

It is thus evident that in many instances the associated micro-organisms can have a beneficial effect on nutrient supply. To properly evaluate this effect and changes that are likely to occur from a manipulation of the microflora, the nutritional conditions under which the experiments are performed must be fully understood and regulated.

B. Influence on plant physiology and metabolism

One might logically expect that if nutrient availability was influenced, plant physiology and metabolism would be indirectly influenced. Clark (1959) refers to the profound physiological effect of micro-organsims on plants. As an example, he refers to soybean chlorosis caused by rhizobium, thus demonstrating that a massive colonization of the root system need not occur in order for the micro-organisms to influence the associated plant.

Subba-Rao *et al*. (1961) found marked differences in the distribution of radioactivity among soluble compounds of the shoot of infected and non-infected plants that were supplied C^{14}-labelled glucose or bicarbonate. *Fusarium* reduced label entering amino acids and increased label in the sugars. This rhizoplane organism thus appears to have a complex influence in the metabolic activity of the whole plant.

Rempe and Kaltagova (1965) claimed that inoculation of sterile oat plants with oat rhizosphere organisms intensified the physiological processes in the plant. In similar experiments with maize, they found that amino acid levels in the sap increased with the inoculation.

Bowen and Rovira (1966) reported that a mixed microbial inoculation to sterile tomato and clover resulted in a 4.4-fold increase in phosphorus translocation from root to shoot. These results again suggest a direct organism influence on plant physiology and metabolism.

Barber (1969) found that microbial inoculation increased nitrate transfer to the shoot by about 20%. Under similar experimental conditions, but using an ammoniacal nitrogen source, the author reported a 10% decrease in nitrogen transfer to the shoot.

Petrenko and Karaseva (1969) stated that the accumulation of carbohydrates in green matter and root crops of sugar beets occurred more rapidly in the presence of bacterial activators.

In a complete study with soybean grown on sterile, sterile-re-inoculated and non-sterile soil, Miller and Chau (1970) reported marked changes in the free ammonia pool. The sterile plants contained 11.2 μmoles/gram of free ammonia, whereas the other two groups contained but 2.0 μmoles/gram.

From this review, it is evident that plant physiology and metabolism is markedly influenced by the presence of micro-organisms. The most commonly noted effects are changes in translocation of inorganic ion and changes in concentration and quality of the free amino acid pool within the plant.

C. Influence on plant growth

Quite logically, the influences on nutrient availability and plant metabolism should be reflected in plant growth. As expected, this has been the most commonly measured microbial influence.

In one of the first reports, Gerretsen (1948) was able to increase growth by 50-200% through a mixed soil culture inoculation to sterile plant systems. He attributed this to improved phosphorus availability.

Akhromeiko and Shestakova (1958) demonstrated a 13 and 26% increase in the growth of oak and ash, respectively, following inoculation with Azotobacter. This can probably be attributed to the production of growth hormones by Azotobacter.

Bowen and Rovira (1961) studied the effect of mixed soil floral inoculum on root, root hair and shoot growth of clover, tomato, phalaris and radiata pine. In all cases, total root length was reduced by the presence of the micro-organism. With the exception of radiata pine, where sterile plants were significantly larger, there was little difference in growth between sterile and non-sterile plants. Under the experimental conditions employed, root hair development was significantly reduced by the presence of the mixed soil flora. They showed that these effects were the result of the action of specific soil micro-organisms that were serially diluted out at higher dilutions. This reduced root hair growth may be the result of antibiotic and growth hormone metabolites.

In contrast to the lack of growth stimulation reported by Bowen and Rovira (1961), Absalyamova (1963) reported that inoculation of sterile soil with isolated rhizosphere complexes resulted in improved plant growth. Rovira (1963) was also able to demonstrate a significant increase in the growth of wheat inoculated with Azotobacter or Clostridium. No effect was noted with a similar inoculation of maize and tomato. In addition, the stimulation to wheat was eliminated with the addition of a complete nutrient solution.

Pantos *et al*. (1964) found that specific micro-organisms stimulated the growth and rooting of poplar cuttings. In the case of *Populus* I-214, this effect was restricted to a mixed culture of 5 organisms, monoculture of *Pseudomonas radiobacter* and a combined *Pseudomonas fluorescens* and *Bacterium candicans*. Very specific and variable results were obtained for other poplar varieties with the different treatments.

Brown *et al*. (1964) carried out a number of experiments where seed, root or soil were inoculated with *Azctobacter* cultures. In pot experiments, crop yields showed an average increase in weight of 11%, but these were generally not significant at the 90% level of probability. In only two cases, where nitrogen was deficient, were significant increases in yield observed.

Rempe and Kaltagova (1965) claimed that inoculation of sterile pots with oat rhizosphere isolates stimulated the growth and development of oats.

Vasantharajan and Bhat (1967) found that soaking cuttings of mulberry in culture filtrates of phytohormone synthesizers hastened rooting. The data from a variety of organism culture filtrates suggest the many cultures (60% of those tested) brought about stimulation of shoot growth, while inhibition of root growth (34% of cultures) was more common than stimulation (26% of cultures).

Leelavathy (1969) noted that several common rhizosphere fungi significantly reduced the growth of grass seedlings. One exception was *Fusarium nivale*, which produced a positive growth response.

Lindsey (1969) grew sterile and inoculated plants in a germ-free environment. For bean, non-dwarf and dwarf tomato, significant increases in yield were noted for a mixed microbial inoculation at day 0. If there was a delay before inoculation, no significant growth differences were noted. They also found that the influence on the shoot was greater than on the root, and increased with time. No significant effect was noted for corn.

Miller and Chau (1970), using a system similar to Lindsey's, found that non-sterile soybean plants yielded significantly greater than the sterile grown soybeans.

Hussain and Vancura (1970) reported that inoculation of maize with organisms capable of producing various growth factors resulted in a significant increase in dry matter production.

In summary, it may be stated that, with the exception of the reports in the Russian literature, there are a limited number of reports of positive growth responses by micro-organism inoculation. Many of these may be traced to the production of various growth hormones by the selected flora. Two of the most thorough reports (Lindsey, 1969; Miller & Chau, 1970) have demonstrated that the micro-organism presence has a positive influence over sterile controls under what is commonly assumed to be adequate nutritional conditions. This influence is most probably associated with increased micro-nutrient availability.

In no instance are there repeated beneficial growth responses through selected mixed or pure culture inoculation to seed, plant or soil when the control is maintained in a non-sterile condition.

III. Materials and Methods

1. Sampling and sample preparation

In each of the following sampling procedures, samples were wrapped in plastic and transported to the laboratory in a cooler maintained at approximately 2-4°C.

A preliminary trial designed to provide some familiarity with problems and methodology was undertaken in late June 1969, by sampling in a "clear-cut" section of a lodgepole pine stand located south and west of the Prairie Creek Ranger Station, in the Rocky Mountain House Forest District. From a range of trees available, a 10 cm, 30 cm and 45 cm tall tree were selected. In addition, a young seedling (12 wks) was selected from some greenhouse container-grown stock and included with the three field plants for processing.

Upon arrival in the laboratory, the root system of each plant was lightly shaken to remove all loose soil. The adhering soil (rhizosphere soil) and root were placed in sterile distilled water and shaken gently by hand to remove all adhering soil. The root sample (rhizoplane) was then transferred to a Waring blendor and blended with distilled water for 5 minutes.

Rhizosphere and control soil samples were shaken in an orbital shaker (2.5 cm orbit) for 10 minutes at 350 rpm to obtain a complete separation of organism aggregates.

In June 1970, a second sampling was undertaken. Young trees of about 12 cm height (Plate 1), growing in a sandy loam soil

Plate 1. Young tree sampling site.

Plate 2. Typical young tree sampled for rhizosphere microflora isolation.

on a cleared power line right-of-way, approximately 48 kilometers north of Whitecourt, Alberta, were collected. This site was chosen because there was no competing vegetation located closer than 20 cm from sample plants (Plate 2).

Rhizosphere and rhizoplane separation of these samples was as suggested by Louw and Webley (1959). Root system and adhering soil were placed in sterile water and rotated 2-3 times. The roots were then removed, care being taken to see that any attached soil material was broken loose. This sample was designated rhizosphere. Roots were transferred to a weighed sterile flask with 4 gm of 1 mm glass beads per 10 ml of water. This sample constituted the rhizoplane.

Larger trees, 27 years old and approximately 6 meters tall (Plate 3) were sampled on a sandy loam site 24 kilometers west of Whitecourt. A root system was excavated so that one surface of the root was exposed as far as it could be traced into the soil (Plate 4). This root was then marked in approximately 30 cm sections. The rhizosphere was sampled by scraping the soil, immediately adjacent to the root in each section, into a sterile 18 mm tube. The control sample for each section was collected 15 cm from the root by scraping freshly exposed soil into another 18 mm tube. The root system was then cut into the marked sections. Rhizoplane samples from these larger roots were prepared in the laboratory by peeling measured bark sections from the root and placing this material in a flask with the 1 mm glass beads in sterile water.

The rhizosphere and control soil samples from the second

Plate 3. Mature tree sampling site.

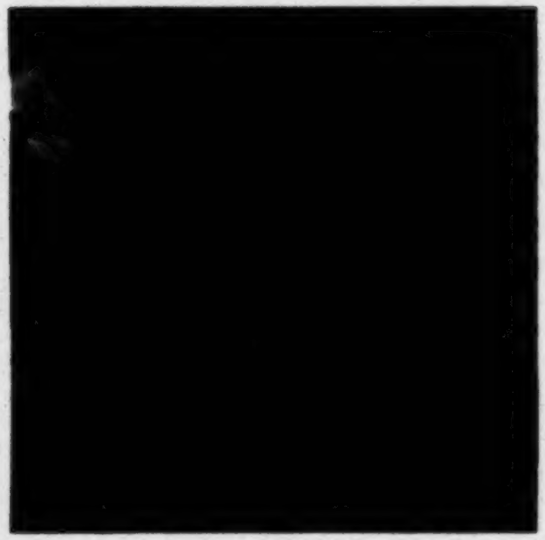

Plate 4. Mature tree root excavation with root exposed.

young tree and larger tree sampling were shaken 5 minutes at 600 oscillations per minute (2.5 cm orbit). The associated rhizoplane samples were shaken 20 minutes under the same conditions.

Shaken samples from all the sampling were serially diluted in sterile distilled water. For all organism counts, three dilutions replicated five times were inoculated to the media and incubated at 20°C for 7 (fungi) or 14 (bacteria and streptomycetes) days before counting colonies with a New Brunswick Impulse Colony Counter.

The media used for counting, storage or characterization of the organisms in this study are fully described in Appendix 1. Bacterial and streptomycete counts were performed by inoculating 1 ml of the appropriate dilution to "pour plates" of Stevenson Yeast Extract. Fungi were counted by spreading 0.1 ml of inoculum on the surface of Peptone-Dextrose-Rose Bengal agar.

All data are expressed as number of organisms per 100 sq. cm. Surface area of soil was determined using the Ethylene-Glycol Monoethyl Ether (EGME) technique (Heilman, Carter & Gonzalez, 1965). Samples were calcium-saturated and ground to pass a 60 mesh sieve prior to treatment with EGME.

Surface area of roots was determined in one of two ways. For small roots, a total length was determined by measurement. The root sample volume was determined by displacement (Baver, 1956) in ethanol. The calculation of surface area was then accomplished by assuming the root segment to be a cylinder and substituting measured and calculated values in the formula for calculation of cylinder volume ($V = \pi r^2 h$) and surface area ($A = 2\pi rh$). A typical calculation is presented in Appendix 2.

With the larger root samples, only measured bark sections were introduced into the rhizoplane sample. In this instance, total surface area was simply calculated using these measurements.

2. Organism isolation and characterization

A. Fungi

From plates prepared in the preliminary trial, 30 fungi were isolated from each of rhizosphere and control soil samples of the 30 cm tree and stored on malt extract agar. Fifteen organisms were similarly isolated from each of the rhizoplane, rhizosphere and control soil of the 45 cm tree. These fungi were subsequently grown on Czapek Solution Agar, Malt Extract Agar and Potato Dextrose Agar for 7 days, at which time they were examined and grouped on the basis of morphological similarities.

B. Bacteria

After counts were completed on four of the plates prepared, organisms were isolated from the fifth plate of a dilution with 30-100 colonies per plate. Sixty bacterial colonies were isolated from each of the three regions (rhizoplane, rhizosphere, control soil) associated with each of the four young trees in the preliminary trial. In the second sampling of young and mature trees, June 1970, a total of 2500 organisms were isolated from the three regions. All organisms were stored on general medium(semi-solid) and, as required, grown up on fresh general medium(broth) for 7 days before inoculation to the various media used in characterization of the microflora.

Surviving organisms from the preliminary sampling and 401 isolates from the control soil and rhizoplane June 1970 sampling were examined for the ability to grow on four nutrient medium. Basal medium, amino acid medi and yeast-amino acid medium were common to all tests, but yeast extract medium replaced yeast-soil extract medium in the June 1970 isolate characterizations. Optical density was determined in a "Spectronic 20" at 600 μm and 1 cm light path after 72-hr growth at 20°C.

Organisms from the 30 cm tree (preliminary sampling) were also gram stained using Huckers modification, and tested for ammonification of casein and ability to solubilize phosphorus.

All surviving organisms from the second young and mature tree sampling (June 1970) were tested for the ability to bring about starch hydrolysis, chitin hydrolysis, casein hydrolysis, phosphorus solubilization and lipolysis of Tween 80. They were also examined for the ability to utilize protocatechuic acid and for the presence of the enzyme phosphatase.

The previously mentioned group of 401 isolates was examined for the oxidase reaction and the enzymes catalase and urease. Growth was also examined under the following pH conditions: 4.6, 5.0, 5.6, 6.0, 6.6, 7.0 and at temperatures of 4°, 20° and 37°C. Cultures were also examined for the ability to bring about ammonification of casein, nitrate reduction and methylene-blue reduction. Oxidative and fermentative metabolism of glucose and lactose were also monitored.

3. Soil chemical analysis

The soil from the young tree site was sampled only in the surface 15 cm because this represented the zone to which root development was predominantly confined. In addition, the surface horizons had been disturbed. The mature trees were found on an orthic podzol and thus sampled by horizon.

The samples were quartered in the laboratory for pH, ammonium and nitrate determination. The remaining sample was then air-dried and stored in quart mason jars.

pH values were determined on a saturated soil paste as outlined by Doughty (1941).

Ammonium and nitrate were extracted by shaking with 2N KCl (10 ml/gm soil) for 1 hr. Ammonium content of the extract was determined by steam distillation in the presence of magnesium oxide (Bremner, 1965). The distillate was collected in boric acid and back titrated with dilute (0.02N) sulfuric acid. Ammonia plus nitrate and nitrite were determined by distillation in the presence of magnesium oxide and Devarda'a alloy.

Available phosphorus was determined on air-dried samples by the acid fluoride method. Five grams of soil were extracted with 1 minute shaking in 25 ml of an extracting solution of 0.03N H_2SO_4 and 0.03N NH_4F. Ten milliliters of the extract was allowed to react with 10 ml of combined vanadate-molybdate reagent. After reaction for 20 min. absorption was determined at 425 μm through a 1 cm light path (Soil Laboratory Analysis, Alta. Soil Survey and Dept. of Soil Sci. University of Alta. Pg. 78d).

Exchangeable cations were extracted from the sample with normal ammonium acetate adjusted to pH 7.0 as outlined in A.O.A.C. (1955). Exchangeable potassium, sodium, calcium and magnesium were determined with the Perkin-Elmer Atomic Absorption Spectrophotometer.

Exchangeable acidity was determined by 0.5N barium acetate adjusted to pH 7.0 and titrated with standardized NaOH, as suggested by Brown (1943).

4. Micro-organisms - Plant growth interactions

In this segment of the work, a number of varied experiments were performed to define technique and requirements of an aseptic culture system before proceeding with organism/plant growth interaction studies.

A. Seed sterilization

An initial experiment was conducted to determine the effect of three sterilants, $HgCl_2$, H_2O_2 and CaOCl, on germination, as well as the ease and frequency with which sterile seed could be obtained. A number of concentrations and times were evaluated. In all subsequent experiments, sterile seed was obtained by stirring seeds in 30% H_2O_2 for 30 seconds and then filtering the seeds out on sterile cheese cloth suspended on a sterile beaker. Seeds were separated and spread out to facilitate complete drainage. All seeds were transferred to petri dishes of plate count agar and germinated at 17°C for 6 days. At this time, normal uniform sterile germinants were planted in a vermiculite and sand soil mix (Appendix 4) adjusted to pH 6.2 with 1N hydrochloric acid.

B. Plant growth conditions

In all experiments, the lodgepole pine were grown over a 16-hr photoperiod at a light intensity of approximately 1000 foot candles. Day temperature was maintained at 21°C, while night temperature was set at 18°C.

Plant aeration within the aseptic chambers was provided by diffusion in the initial experiments. This was replaced by a pressurized air steam, which was sterilized before entry to each chamber by filtration through Millipore GS 0.22 μ pore size filters held in 25 mm Swinnex holders.

The plant density in all experiments was adjusted so that a minimum of 41 cc (2.5 cubic inches) of rooting volume was available per plant (Endean, 1971).

Nutrients was provided by watering with various concentrations of nutrient solution (Hocking, 1971).

In experiments where a portion of organic rather than all inorganic nitrogen was supplied, equivalent amounts of casein-nitrogen were added to replace the ammoniacal- and nitrate-nitrogen removed. All other elemental forms were identical to those in Hocking's solution (Appendix 5). The quantity of added nutrients required to any point in the growth period was calculated by developing a hypothetical growth curve, based on published data, and then assuming an optimal plant nitrogen concentration for this plant (Appendix 6).

The pure culture isolates used as inoculum in the organism/plant interaction studies were grown for 4 days on general media before four drops were added around the root collar of one of the plants. A mixed soil culture was grown up on a basal salts medium containing avicel, casein and soluble starch (Appendix 1). In this inoculum preparation, 1 gram of soil was added to 100 ml of the media. This was grown in shake culture at 15°C for 7 days. One milliliter of this mixture was then transferred to fresh medium and the growth condition repeated. On the third transfer, the mix was grown for 4 days and was then introduced to the sterile system in the same manner as the pure cultures.

At the end of a selected growth period, plants were harvested and shoot and root length measured. After drying for 48 hr at 75°C, shoot and root dry weights were also determined.

The establishment and number of organisms within the various rhizospheres was determined by aseptically removing soil and plant root from the growth chambers. Adhering soil was removed from the roots as outlined previously. This was serially diluted and plated by spreading on general media for counting. Sterility checks for sterile control plants were made by placing shoot, root and soil mix of different plants on plate count agar and incubating for 14 days at 20°C. Where there was only one plant per chamber, only the soil mix was plated for sterility checks.

IV. Results and Discussion

1. Preliminary investigations

This study was designed to develop some experience with methodology and to indicate areas for improvement within the proposed plan. Table 4 shows results of the bacterial counts. The expected rhizosphere effect is demonstrated but is not of the magnitude evident with agricultural crops (Alexander, 1961), and is less than the values reported by Timonin (1966) and Hocking and Cook (1972) for lodgepole pine reared under artificial conditions. Shipman (1957), in a study of yellow poplar, reported that R/S values of 2.3 or greater were required to be significant at the 99% level of significance. This would suggest that the difference with the 10 cm tree is not significant. The low rhizosphere influence reported here may be real, or a result of sampling error. Samples were collected in a "clear-cut" area, in which there was vigorous growth by many plant species. As a result, the comparison between lodgepole pine rhizosphere soil and control soil may really be a comparison of rhizosphere soils for lodgepole pine and some other plants present on the site. For a detailed comparison, some site with a minimum of competing vegetation is required.

As expected, the number of organisms within the rhizoplane are more numerous than in the rhizosphere. No real comparison is possible because in the rhizoplane counts are based on oven-dry weight of root and, in the rhizosphere, they are based on oven-dry weight of soil, and thus on material of differing bulk density. In addition, the soil is porous and may have a very large surface area,

Table 4. Bacterial Numbers/gram Oven Dry Wt. of Sample (x 10^5)

Sample	Control Soil	Rhizosphere Soil	R/S	Rhizoplane	% of Control
Seedling	370	1990	5.4	2690	725
Sapling 10 cm tall	41	68	1.7	626	1520
Sapling 30 cm tall	12	103	8.6	183	1520
Sapling 45 cm tall	158	513	3.2	13	--

whereas a root is solid and has only a single external surface. For a proper comparison, the numbers must be placed on a common base, such as surface area, as was suggested by Harper (1950).

Fungal numbers are reported in Table 5. Only in the larger, more mature sapling was a positive fungal rhizosphere effect noted. This can be the result of the sampling procedure mentioned earlier or perhaps of inhibition within the root-influenced soil.

Table 5. Fungal Numbers/gram Oven Dry Wt. of Sample (x 10^3)

Sample	Control Soil	Rhizosphere Soil	R/S	Rhizoplane	% of Control
Seedling	2140	68	-	1170	55
Sapling 10 cm tall	113	97	-	100	88
Sapling 30 cm tall	45	84	1.9	lost	-
Sapling 45 cm tall	126	635	5.0	50	40

Another possibility is that this result is the product of the isolation procedure selected. Parkinson and Thomas (1965) have demonstrated that the soil dilution procedure selects for the heavily sporing fungi. Within the rhizosphere, fungi exist primarily in the vegetative state while, within the soil, 70-90% of the colonies isolated develop from spores (Agnihothrudu, 1955). Counts with the dilution technique would thus not provide a proper evaluation of fungal activity. As support for the validity of these data, however the observations of Parkinson, Taylor and Pearson (1963), demonstrating increased frequency of isolation with increased root age, may be cited.

While the validity of the quantitative data is open to question, there are strong indications of a selective root influence within the rhizosphere soil. Of the fungal isolates from the 30 cm tree that were morphologically grouped after examination on four media, fourteen occurred exclusively in the control soil and eight exclusively in the rhizosphere. Another nine organisms were common to both environments.

When 15 isolates from each of rhizosphere, rhizoplane and control soil of the 45 cm tree were compared in a similar manner, it was observed that about one-third were common to all regions. Another 50% of the isolates were unique to each of the environments and the remainder were shared by two of the three sampled regions. This was not unexpected and is similar to earlier reported results (Katznelson _et_ _al_., 1962b; Timonin, 1966).

The nutritional groupings of the isolated bacterial flora are presented in Figure 1. There are no marked changes in the requirements for maximal growth by the organisms isolated from the various regions. The graphical presentation of data suggests that generally there is a decrease in percentage of organisms capable of maximum growth on basal medium within rhizosphere and rhizoplane. Alternately, there is an increase in the proportion within the two root influenced regions exhibiting maximum growth on amino acid medium. The trend found within the root-influenced regions was rarely more strongly expressed within the rhizoplane.

In all observations for rhizosphere and rhizoplane, the

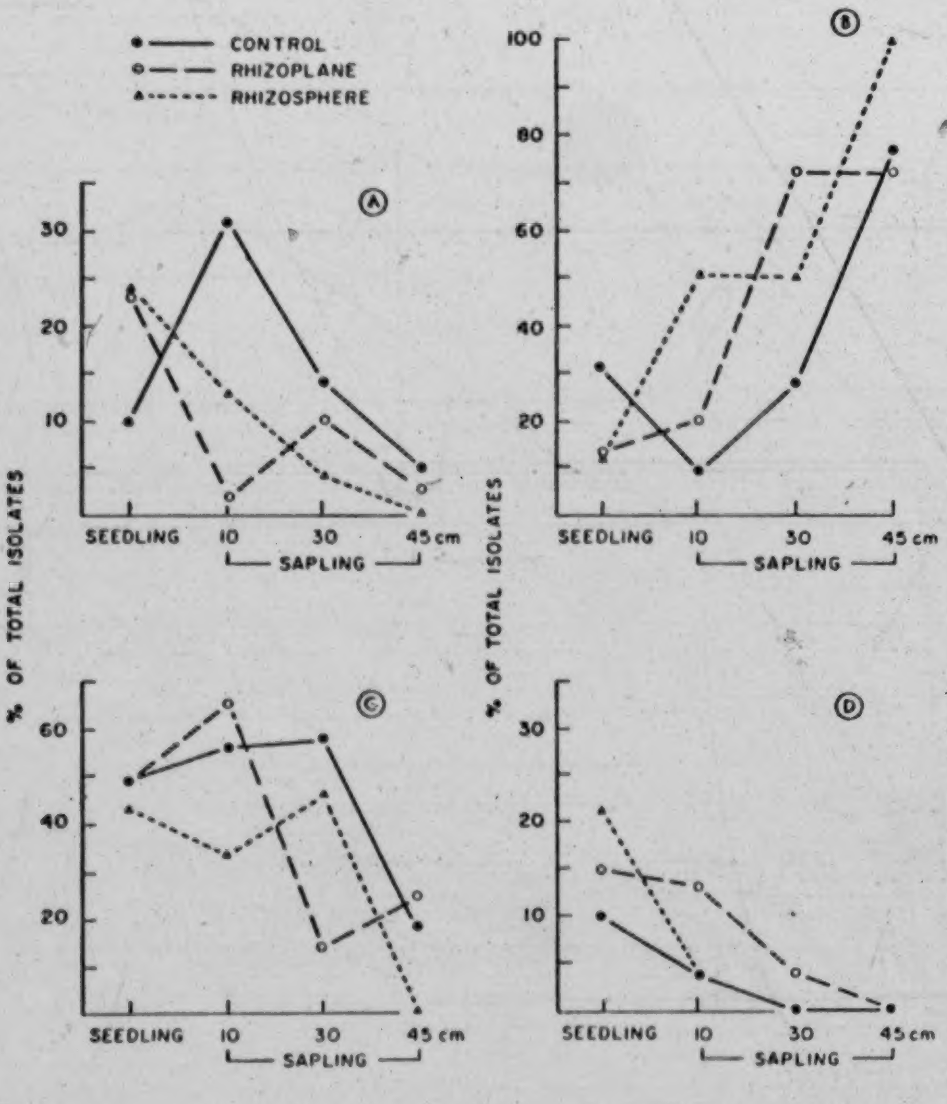

Figure 1. Proportion of isolates from preliminary sampling exhibiting maximal growth on A) Basal medium, B) Amino acid medium, C) Yeast extract - amino acid medium and D) Soil-yeast extract medium.

maximum differences from control soil samples occur with the 10 and 30 cm tree. The observations here are probably the most realistic, since the seedling was grown in an artificial environment while the 45 cm tree was rooted primarily within rotten wood.

The small difference between rhizoplane, rhizosphere and control soil suggests the controls may be root-influenced, as a result of sampling problems, or that the root exerts a miniumum influence on the soil. Another possible explanation, supported by Gyllenberg's (1957) observations, is that the long period of lodgepole pine growth on this site has led to a soil microflora similar to that of the typical rhizosphere.

Organisms from the 30 cm tree were selected for further characterization (Table 6) because they demonstrated the maximum selectivity based on the nutritional groupings. As is evident, the almost universally reported stimulation of gram negative-bacteria within the rhizosphere does not occur. Since the control soil samples have a high proportion of gram negatives, the immediate reaction is to suggest the control must be root-influenced. The proportion of gram-negatives found in control soil is however, similar to that observed in several other Alberta forest stands (Dangerfield, unpublished data).

The phosphate solubilizers appear to be stimulated within the rhizoplane, but not within the rhizosphere. Selective stimulation of organisms with this capability has been reported for barley (Katznelson _et al._, 1962a), clover, ryegrass, perennial ryegrass, and wheat (Sperber, 1958) and supports this observation. Unfortunately, for every reported stimulation, there are probably two reports of a lack of selective stimulation.

Table 6. Characteristics of the Flora Isolated from the 30 cm Tree

Sample	Number of Isolates	% Gram-Negative	% Ammonifiers	% Phosphate Solubilizers
Control	55	88	44	9
Rhizosphere	60	86	17	8
Rhizoplane	57	87	46	28

Ammonia production from casein is suppressed within the rhizosphere and unaffected within the rhizoplane. Organisms with this capability are almost universally stimulated within the rhizosphere of agricultural crops (Katznelson, 1946; Timonin and Lochhead, 1948; Katznelson and Rouatt, 1957a; Neal et al., 1970). In contrast to the agricultural crop, Ivarson and Katznelson (1960) found this group of organisms stimulated within yellow birch rhizosphere only in 2 of 5 seasonal sampling dates. Neal et al. (1964) reported for Douglas-fir that the proportion of ammonifiers decreased from that of the control soil. Because of the questionable nature of the control samples in this instance, the lodgepole pine influence cannot be properly evaluated.

With the experience gained in the preliminary study, certain criteria for the sampling site were laid down. These included, as a very high priority, the necessity of a minimum of competing vegetation and the need for a coarse textured (sandy loam) soil that would reduce sampling error in the division between the various zones, control soil, rhizosphere soil and rhizoplane.

2. Rhizosphere of young and mature lodgepole pine

A. Determination of root surface area

Organism counts are performed on soil samples to compare organsim density. The comparison of soil and rhizoplane counts is invalid because the soil organisms are spread randomly throughout a porous matrix and the rhizoplane organisms are located only on the surface of the root cylinder. A proper comparison of the organism densities requires expression of the data on a realistic common base. The only logical choice is surface area. To date, this base of comparison has not been used for rhizosphere studies.

Because of the damage to the young tree roots that resulted from the shaking with glass beads for rhizoplane counts, actual root surface area determinations were impossible. This problem was circumvented by establishing a relationship between oven-dry weight of roots and surface area. To do this, 13 additional seedlings, exhibiting shoot development similar to those used for organism counts, were characterized as to root weight, average root radius and surface area. Surface area was calculated by making certain assumptions and by determining root volume by displacement (Baver, 1956) in ethanol. The rationale for the choice of ethanol and a typical calculation are outlined in Appendix 2. Eight of the 13 root system areas were also determined by displacement in water plus a few drops of Tween 80, with closely comparable results. These data are compiled in Table 7 and in Figure 2. As would be expected

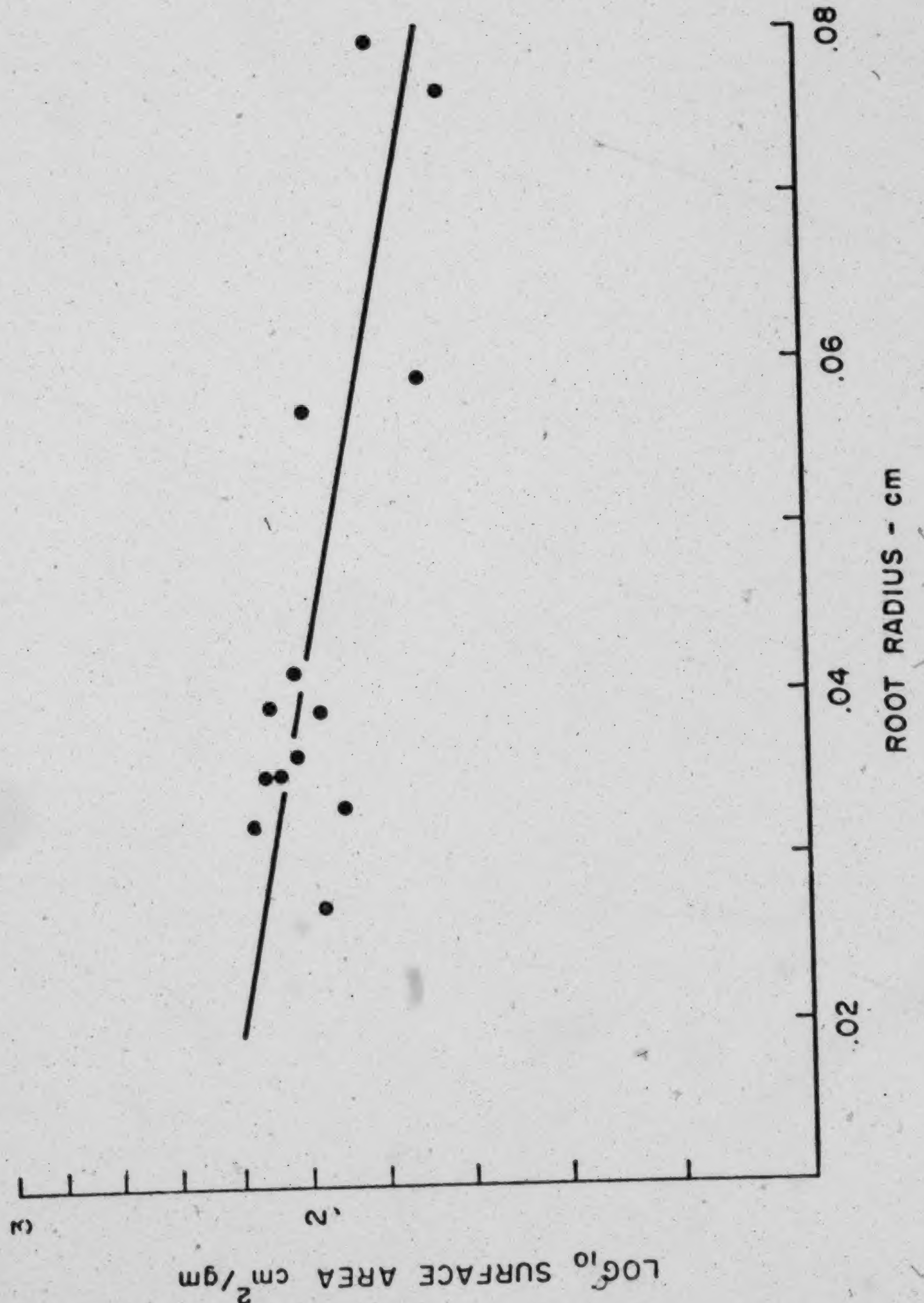

Figure 2. Logarithmic plot of surface area of roots of different radius.

Table 7. Root Surface Areas

		Surface Area (sq cm)			
Sample #	Radius cm	Ethanol	H20 plus Tween 80	Oven Dry Wt.	S.A/gm*
1	.057	24.2	24.1	.253	96
2	.079	10.4	10.6	.172	61
3	.036	29.2	30.7	.288	102
4	.035	34.0	33.6	.295	115
5	.041	39.0	40.8	.369	106
6	.039	21.1	20.4	.173	122
7	.032	26.5	25.8	.188	141
8	.035	14.4	14.5	.115	126
9	.033	23.1		.30	77
10	.039	40.5		.46	88
11	.027	26.0		.25	104
12	.059	48.0		1.0	48
13	.076	20.8		.49	42

* Surface area expressed as sq cm

Average of all values 95.5/gm

Average of all values with root radius less than 0.05 cm = 109 sq cm/gm

Range of values = 42 - 141 sq cm/gm

with data developed with the inclusion of some assumptions, the results are highly scattered. In addition, because of the inverse relationship between surface area and radius, the surface area increases rapidly as the root radius decreases.

Because the seedlings used in the determination of total counts had small roots and the larger root surface area tended to decrease counts per given area, all calculations of counts per 100 sq cm for the seedlings were based on an average surface area of 109 sq cm/gm. This value was obtained by averaging the surface area of all root systems with an average radius of 0.05 cm or less (Table 7). In the final analysis, this decision was of little consequence in the demonstrated rhizoplane effect.

B. Total Organism Counts

Counts from the seedlings sampled are presented in Figure 3 and Appendix 7. Total counts for the control soil are similar to those found in other lodgepole pine stands in Alberta (Dangerfield, unpublished data). The lack of vegetative interference, the coarse texture, the reproducibility of the control counts and the comparable values reported on other sites all suggest a satisfactory control. The rhizosphere effect is distinct and certainly of greater magnitude than that found in the preliminary investigation. This again indicates a more representative sampling. Because competing vegetation is lacking, it would be suggested that the soil is of a poorer nutritional quality than that found in the preliminary study. This could account for the increased rhizosphere effect (Runov and Zhdannikova, 1960). Whatever the explanation, the values reported here are greater than those reported by Timonin (1966) for artificially grown seedlings.

As expected, the greatest numbers of organisms are found

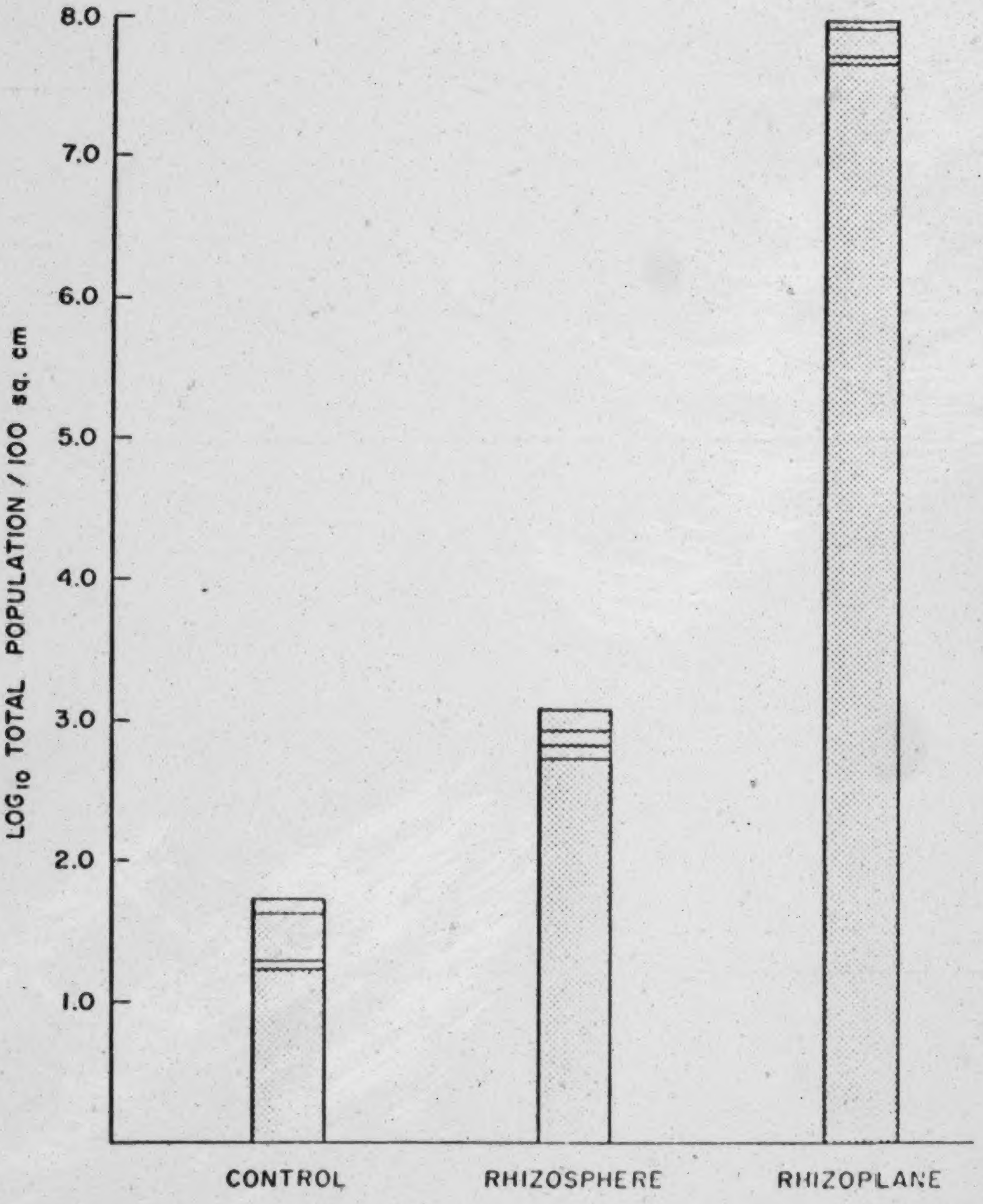

Figure 3. Replicate values of total bacterial counts from sampling of four young trees.

in the rhizoplane. The magnitude of the change is, however, quite unexpected. Equally important is the reproducibility of these observations. Similar trends are observed for the mature tree sampling (Figure 4 and Appendix 7). Here, the total counts decrease significantly only below 120 cm and the difference between control and rhizosphere soil becomes significant only after root diameter drops below about 0.25 centimeters. Again the differences between control soil and root surface (rhizoplane) are extremely great. As reported in the methods section, the root surface area for these samples was calculated by measuring the dimensions of root sections used in the sample preparation and should thus be subject to limited error. The R/S values in the mature tree sampling are similar in order of magnitude to those found for the seedling. In addition, the difference between seedling and mature trees is reduced as the root diameter of the mature tree decreases. This would suggest that the errors involved in calculating the seedling root surface area are at least tolerable.

Total streptomycete counts on the mature tree samples were arrived at by counting all colonies having a firm consistency and a powdery surface (Figure 5). The trend is the same as that for the total count. The only difference is in the order of magnitude and in the proportion of the total (Table 8). The streptomycete proportion increases with depth in control soil and to a lesser extent in the rhizosphere soil. Timonin's data (1935) for a number of virgin profiles in Manitoba indicated that streptomycete proportion did not increase with depth. Within the rhizoplane, the

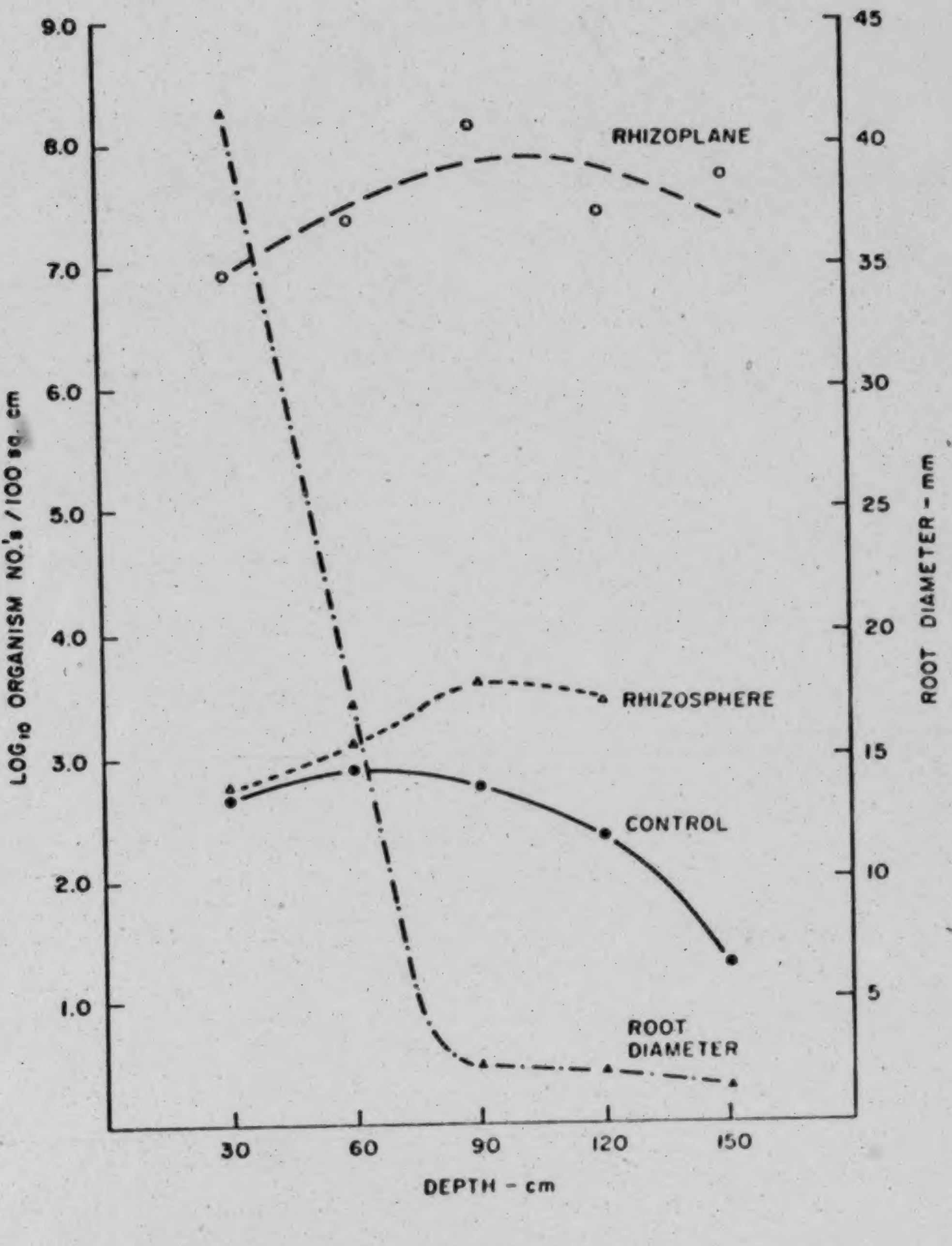

Figure 4 - Variation in total bacterial plus streptomycete population with depth, root diameter and habitat.

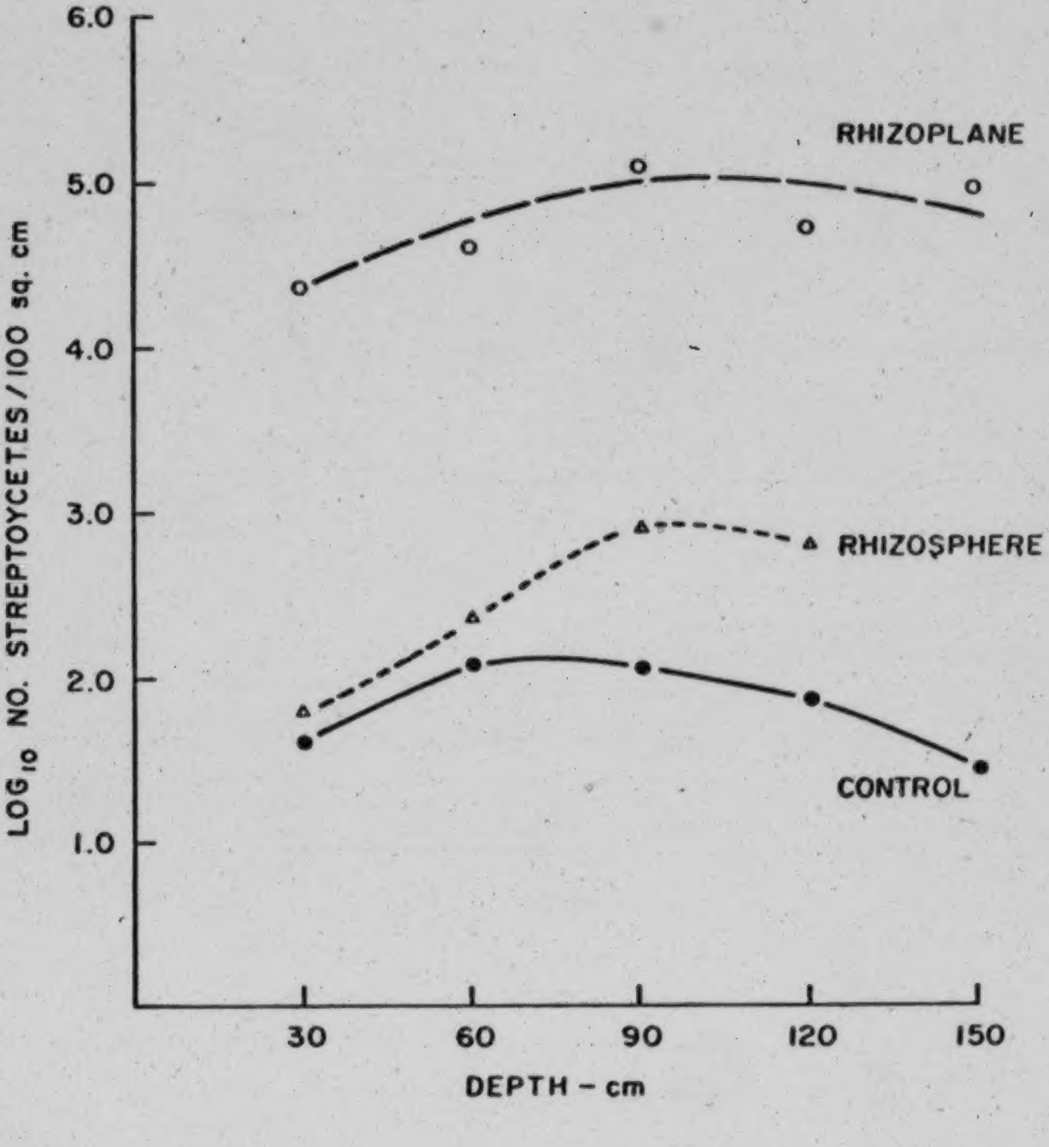

Figure 5. Variation in number of Streptomycetes with depth and habitat.

Table 8. Streptomycete Population Component as Influenced by Depth & Habitat

Sample No.	Depth cm	Control % of Total	Rhizosphere % of Total	R/S	Rhizoplane % of Total	R/S
$1T_1$	30	9	9	1.35	28	5.5×10^2
T_2	60	14	18	1.8	18	3.5×10^2
T_3	90	20	20	7.1	11	1.2×10^3
T_4	120	32	23	8.1	14	7.4×10^2
T_5	150	14			19	3.6×10^2

per cent streptomycete decreased with depth. It thus appears that under maximum root influence the streptomycetes are stimulated to a lesser extent than the bacterial component. A number of workers (Alexander, 1961; Katznelson, 1959) also observed that actinomycetes are stimulated to a lesser extent than are bacteria.

In summary, the rhizosphere phenomenon is more weakly expressed on large diameter than on small feeding roots. This would be expected, since the small feeding roots are responsible for all root exudation. The magnitude of the rhizoplane activity, as indicated by total organism count, is surprisingly large. In addition, the activity within the rhizoplane becomes progressively larger with decreasing root diameter, again indicating the more vigorous activity associated with the feeding roots. The streptomycete component of the flora was stimulated to a lesser extent in the rhizoplane than was the bacteria component.

C. Organism characterization

A total of 2500 organisms were initially picked from the variety of soil, rhizosphere soil and rhizoplane organisms counted. Many of these did not survive the repeated transferring required to check organism purity and to maintain the cultures (Table 9). Survival here is much lower than that reported by either Neal *et al.* (1964) or Oswald and Ferchau (1968), who reported 95 and 69% survival, respectively, for tree rhizosphere isolates. If some of these transfers could have been avoided, this relatively low survival might have been improved. The use of a different storage medium could have been beneficial. The data suggest there was a tendency for decreased survival from control soil to rhizoplane. Many of the high losses within the rhizoplane were isolates from the mature trees. For this reason, the mature tree isolates received only a limited characterization.

Table 9. Organism Survival

Sample	Number Isolated	Number Surviving	% Survival	Range For Various Groups
Control Soil	970	589	61	43-73
Rhizosphere Soil	658	385	58	25-70
Rhizoplane	872	435	50	18-78

The results from mature tree isolate characterization are presented in Figures 6 and 7. Because of the limited number of organisms characterized in all of the regions but the control soil at 30, 60, 90 and 120 cm, absolute values are of little significance and only relative relationships between rhizoplane, rhizosphere and control soil can be considered.

The control soil isolates do, however, provide a realistic measure of absolute fluctuations with depth in the absence of root influence. Here the proportion of chitinolytic, amylolytic and proteolytic (Figure 6) organisms increased with depth to a maximum at 90 cm and then decreased again. This pattern is identical to that for total number of organisms. At all depths, with the exception of the 150 cm sample, the capability to handle these substrates is such that proteolytic > amylolytic > chitinolytic. In contrast to the trend indicated for the control samples, the proportion of organisms which are chitinolytic, amylolytic and proteolytic within rhizosphere and rhizoplane samples reaches a minimum at the 60 to 90 cm depth and then increases with continued depth. This would suggest a definite inhibitory root influence as related to these characteristics within the medium depth range studied and a possible increase within the feeding root zone. While no explanation for the very similar reaction in selectivity of these three characteristics is offered, a close relationship between proteolytic, chitinolytic and amylolytic capability also has been observed within Douglas-fir forested soils (Dangerfield, unpublished data). Of the non-fermentative gram-negative bacteria examined by Gilardi (1973), only the Flavobacterium were both amylolytic and proteolytic, with the proteolytic capability being more frequently expressed. In addition, only one species of Pseudomonas, Pseudomonas stutzeri, was amylolytic. Loutit, Hillas

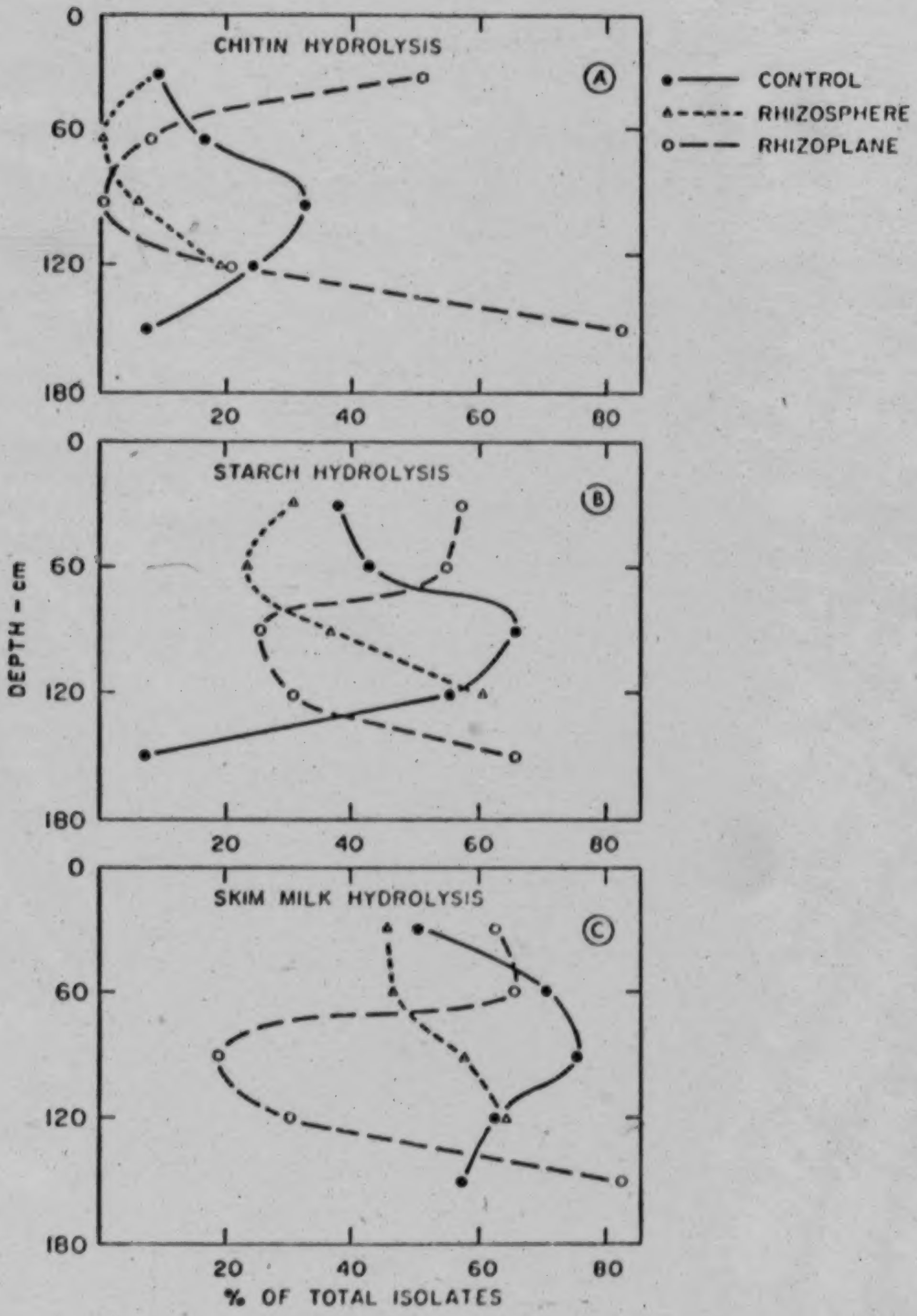

Figure 6. Proportion of mature tree rhizoplane, rhizosphere and control soil isolates from different depths capable of A) Chitin hydrolysis B) Starch hydrolysis C) Skim milk hydrolysis.

and Spears (1972) found that the Flavobacterium represented only 8% of a radish rhizosphere flora. Flavobacterium proportionately decreased within the rhizosphere of Sesleria caerulea (Leval and Remacle, 1968). It is also frequently reported (Clark, 1949) that the Bacillus, which are proteolytic and amylolytic, are suppressed within the rhizosphere.

The ability to bring about phosphate solubilization is found to decrease with organisms isolated at greater depth (Figure 7). In this case, however, the data for rhizosphere and rhizoplane isolates suggest this capability is selectively enhanced. This supports the preliminary study observation of increased phosphate solubilizers within the rhizoplane.

The ability to bring about the lipolysis of Tween 80 and the presence of the enzyme phosphatase fluctuate less dramatically with depth and between sampling zones (Figure 7). These characteristics appear to be little influenced by the root presence.

Because of the limited number of organisms available from the mature-tree-root-influenced samples, no further characterizations of these organisms were attempted and all subsequent discussions are concerned with the characteristics of the young-tree-root-influenced isolates.

Initially, 458 organisms from control soil, rhizosphere soil and rhizoplane of the young-tree sampling were characterized, using six tests (Table 10). The data for the surface sampling (0-30 cm) in the mature tree site are included to indicate that, based on these six characteristics, there are little differences in

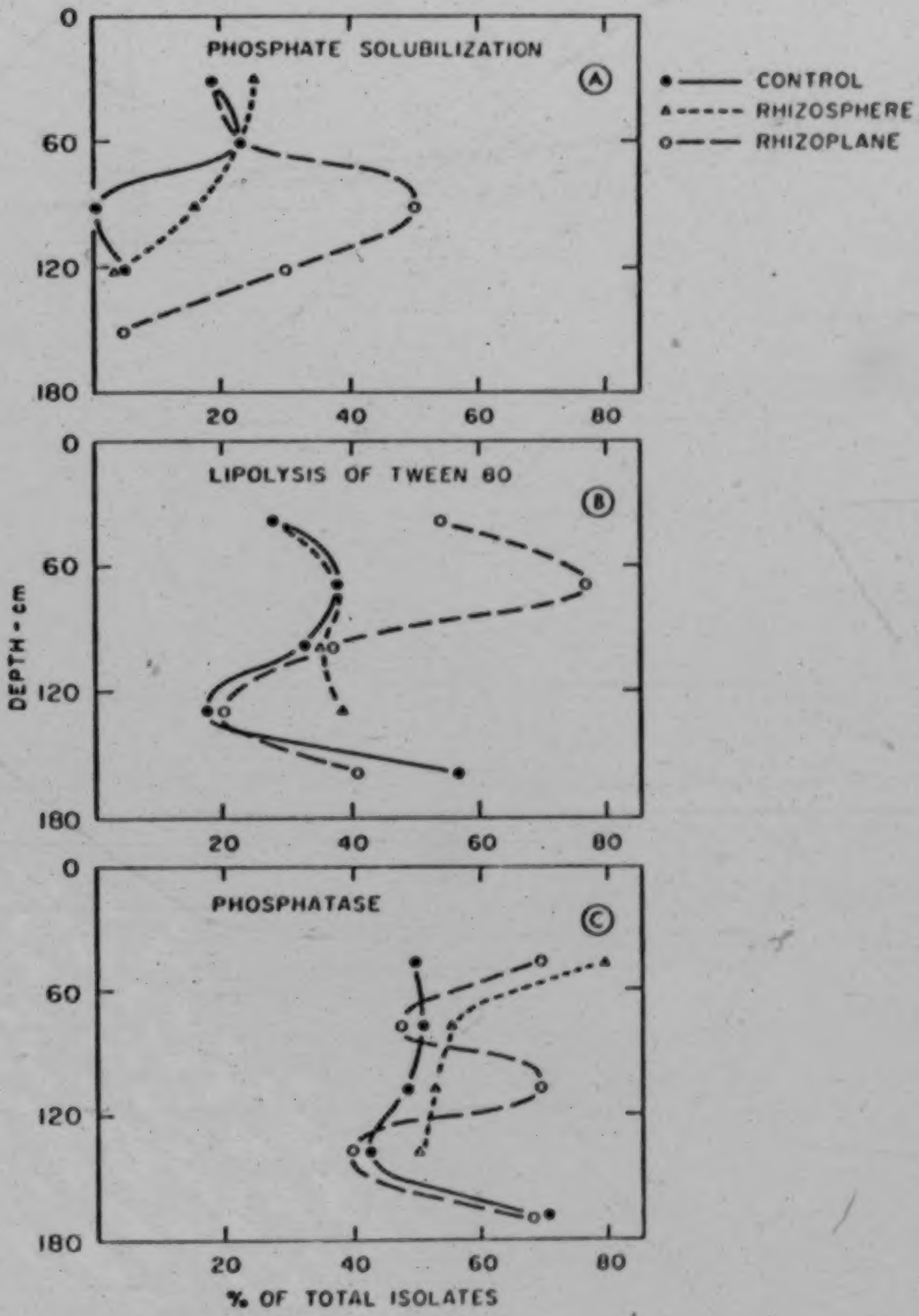

Figure 7. Proportion of mature tree rhizoplane, rhizosphere and control soil isolates from different depths capable of A) Phosphate solubilization and b) Lipolysis of Tween 80 and C) Posessing the enzyme Phosphatase.

Table 10. Percentage of Isolates from Young-Tree Sampling Demonstrating Positive Reaction for Six Metabolic Capabilities.

Sample	Lipolysis Tween 80	Starch Hydrolysis	Phosphatase	Skim Milk* Clearing	Phosphate** Solubilization	Chitin* Hydrolysis
Controls (Young tree)	25	44	40	65a	26a	8a
Controls (Mature Tree) 0-30 cm	28	37	10	50	21	9
Rhizosphere	25	33	28	41b	39b	9a
Rhizoplane	37	33	34	37b	44b	24b

* Means followed by different letters are significantly different at the 99% probability level.

** Means followed by different letters are significantly different at the 95% probability level.

control soil characteristics. As noted for the mature tree, the rhizosphere selectively stimulates phosphate solubilizing organisms (99% level of significance) and inhibits the proteolytic microflora (95% level of significance). There also is an indication of suppression of the amylolytic flora but this is not significant. In contrast to the mature tree, where chitinolytic, proteolytic and amylolytic flora fluctuated together and were suppressed, the seedling significantly (99%) stimulates the chitinolytic flora.

It has been commonly reported (Timonin and Lochhead, 1948; Balicka, 1958; and Rouatt, 1959) that proteolytic flora are stimulated within the rhizosphere. The only really comparable demonstration of suppression is the observation by Remacle (1963) that plants growing in mor soil did not stimulate this floral component, whereas those in mull and moder did. Pine certainly could be classed as a plant that grows in a mor type soil.

Growth achieved following drop inoculation to a variety of media was monitored spectrophotometrically after 72 hr at 20°C. The results for control and rhizoplane isolates of seedling sample 1 are presented in Figure 8. It is apparent that rhizoplane organisms achieve a lower cell density and presumably grow more slowly on all media examined than do the control organisms. The selective pressure within the rhizosphere cannot be explained in any way by a more rapid growth by rhizoplane organisms. The observation of slower growth rates by rhizoplane organisms is different from the observations (Lochhead, 1940; Rovira, 1956c; Rouatt and Katznelson, 1957) made for isolates from a number of agricultural crop rhizospheres.

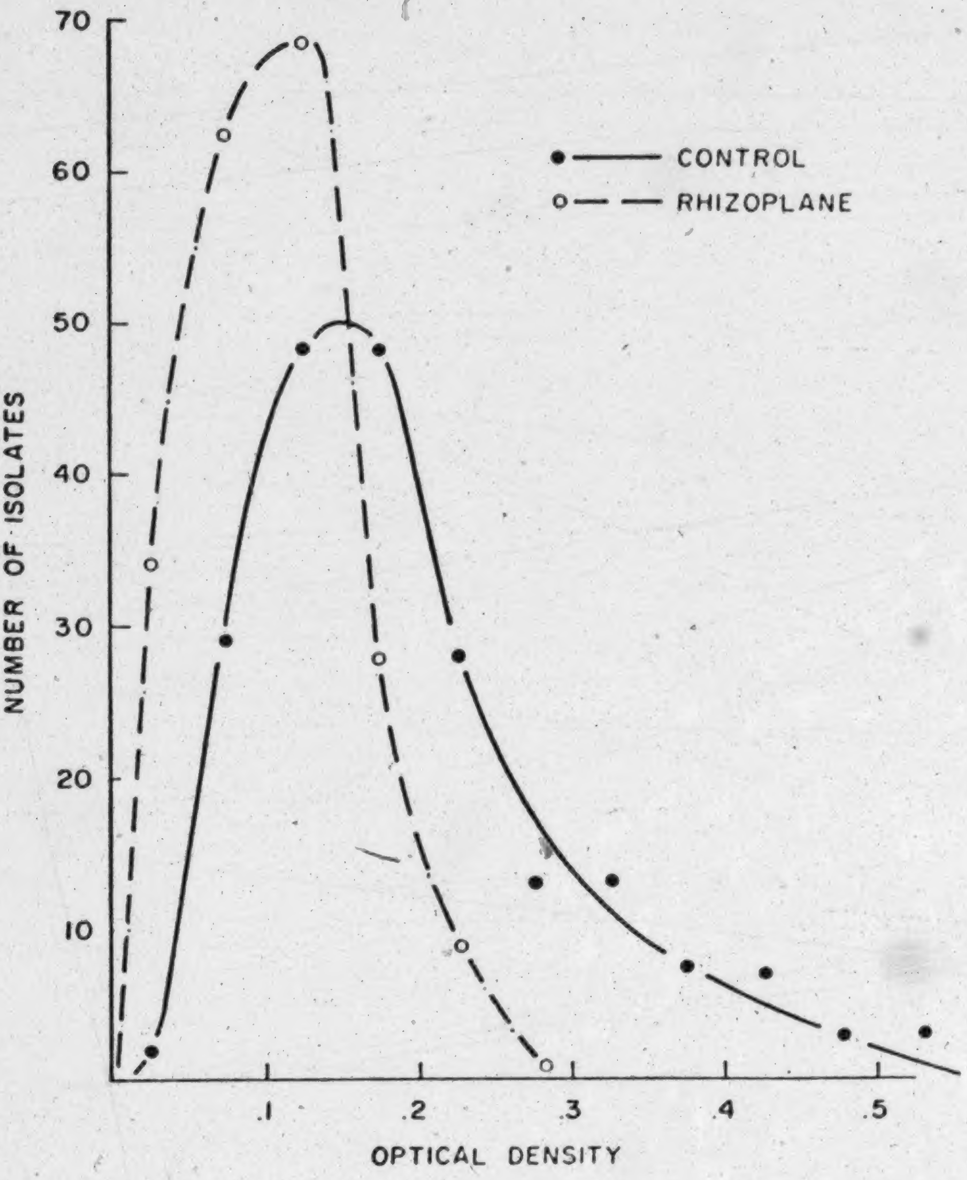

Figure 8. Distribution of Optical Density readings after 72 hr. growth of rhizoplane and control soil isolates on all nutritional media.

This is doubly interesting since a number of the original isolates have been lost, among which probably were a large number of less active isolates. If this were the case, the remainder would logically have been biased toward nigh activity and growth.

Many soil organisms fail to produce acid from single carbohydrates (Skyring and Quadling, 1969). Sundman (1970) reported that "of the attributes for carbohydrate utilization, only those concerning aerobic use of disaccharides showed significantly uneven distribution among soil bacterial populations studied". Thus to reduce the number of carbohydrates utilized in the final testing, a number of organisms from control and rhizoplane were randomly selected and characterized for the reaction on a number of disaccharides (Table 11). The amylolytic and proteolytic capabilities were also monitored to serve as an indicator of normal population distribution. The reduction in amylolytic capability is not significant, while proteolytic capability is significant (99% level of significance). The failure to produce acid (Skyring and Quadling, 1969; Gyllenberg and Rauramaa, 1966) is again noted. This appears to be more noticeable with the rhizoplane isolates and is particularly evident with the disaccharide lactose. Bowie, Loutit and Loutit (1969), using Skerman's key for the identification of aerobic soil heterotrophs, required only two carbohydrates, glucose and lactose, for taxonomic implementation. Many Pseudomonads are capable of acid production from lactose while few, if any, of the Achromobacter and Flavobacterium produce acid from lactose (Cilardi,

Table 11. Metabolism of Various Carbohydrates By Randomly Selected Control Soil and Rhizoplane Isolates.

Assay	% of Control Soil Isolates (120)		% of Rhizoplane Isolates (90)	
	Acid pH	Alkali pH	Acid pH	Alkali pH
Glucose	90	2	91	4
Sucrose	79	10	57	31
Cellobiose	63	28	64	22
Lactose	67	23	24	67
Maltose	74	10	67	19
Amylolytic Flora	52		37	
Proteolytic Flora	73		35**	

** significantly different at the 95% probability level.

1973). All three groups have a high incidence of acid production from glucose. Loutit, Hillas and Spears (1973) have reported that these three genera of organisms, *Achromobacter*, *Flavobacterium* and *Pseudomonas*, represent about 27, 6.6 and 12% of the isolates of radish rhizosphere grown on a molybdenum fertilized Hasting soil. It is worth noting that the *Achromobacter* are also rarely proteolytic or amylolytic (Gilardi, 1973; Breed, Murray and Smith, 1957). *Achromobacter* proportionately increased within the rhizosphere of *Sesleria caerulea* (Leval and Remacle, 1968). Thus, the reduction of the rhizoplane population ability to produce acid from lactose and bring about the hydrolysis of casein and starch could be explained by a proportionate increase of the *Achromobacter*.

After reducing the number of carbohydrates utilized in the final testing, a total of 401 isolates randomly selected from the control soil and rhizoplane isolates were characterized, using 26 tests considered to be of ecological significance, and which also allowed a comparison with data from other rhizosphere studies. Of the 26 tests performed (Table 12), five (Positive Amino Acid Response, Positive Yeast Extract-Amino Acid Response, Ammonification, Growth at 3-4°C, Urease) produced a difference significant at the 95% level and two (Amylolytic, Alkali from Lactose) were significantly different at the 99% level. The response to amino acids is similar to the observation reported for many agricultural crops (West and Lochhead, 1940; Timonin and Lochhead, 1948; Rouatt *et al*., 1960) and non-agricultural plants (Katznelson *et al*., 1962b; Leval and Remacle, 1968). The reduction in ammonifiers is similar to that

Table 12. Results of Physiological Tests on Control Soil & Rhizoplane Isolates

Test	% of Control Soil Isolates (54) A[1]	(38) B[1]	(58) C[1]	(54) D[1]	(204) Avg	% of Rhizoplane Isolates (50) A[1]	(72) B[1]	(56) C[1]	(19) D[1]	(197) Avg
Oxidase (+)	19	42	9	9	18	34	36	29	21	32
Catalase (+)	82	68	78	69	74	82	63	52	26	61
Positive Yeast Extract Response	28	24	31	35	30	52	56	30	32	45
Positive Amino Acid Response	11	13	10	22	14	24	28	21	26	25*
Positive Yeast Am.A Response	57	63	46	57	55	76	82	57	74	73*
Maximum Gr. on Basal media	35	37	50	39	41	18	15	41	26	24
Ammonification(+)	28	55	36	26	35	12	10	20	26	15*
N-Red(+)	19	16	17	33	22	10	0	23	5	10
Acid from Glucose	37	39	66	67	53	68	60	63	89	65
Alkali from Lactose	50	29	52	44	45	76	86	70	89	79**
Growth 37°C	48	87	64	57	62	84	65	52	53	65
Growth 3-4°C	96	89	90	93	92	32	42	79	84	62*
Methylene Blue Reduction	91	79	84	81	85	88	79	59	63	74

Table 12. Results of Physiological Tests on Control Soil & Rhizoplane Isolates (Cont'd.)

Test	% of Control Soil Isolates					% of Rhizoplane Isolates				
	(54) A[1]	(38) B[1]	(58) C[1]	(54) D[1]	(204) Avg	(50) A[1]	(72) B[1]	(56) C[1]	(19) D[1]	(197) Avg
Urease (+)	30	42	26	19	28	10	8	20	0	11*
Chitinase(+)	11	42	5	4	13	2	7	13	0	7
Lipase(+)	33	29	45	44	39	70	51	45	42	53
Phosphatase(+)	30	42	31	22	30	60	36	32	47	42
Protease(+)	65	76	78	67	71	70	60	43	26	54
Amylase(+)	13	21	14	15	15	2	0	7	0	3**
PO_4-Sol(+)	20	8	12	35	20	12	7	25	5	13
Growth pH 4.6	72	53	86	69	71	76	57	57	53	61
5.0	82	66	91	83	82	86	65	68	74	72
5.6	91	89	100	89	93	90	94	84	100	91
6.0	96	92	100	90	96	96	97	89	100	95
6.6	96	97	100	100	100	100	100	100	100	100

* Significant at 95% level

** Significant at 99% level

[1] Represents 4 individual trees sampled

reported by Neal et al. (1964) for Douglas-fir mycorrhizal root rhizosphere, and Rambelli (1967) for radiata pine rhizosphere isolates. It is in direct contrast to the frequently reported stimulation within the rhizosphere of agricultural crops (Katznelson, 1946; Timonin and Lochhead, 1948; Rouatt et al., 1960). The reduction in ammonifiers is related to the reduction in proteolytic flora within the rhizoplane, which is not significant with this group of organisms but is significant with the organisms characterized in the other groupings.

The reduced growth at 3-4°C by rhizosphere isolates was initially thought to be related to the slower growth rate of these organisms. This was checked by growing up organisms at 20°C, measuring the optical density and subsequently incubating these cultures at the 3-4°C temperature. Optical densities were re-measured at 1 and 2 weeks, but no increase in optical density was recorded for those organisms that did not grow in the first trial. The only explanation that seems reasonable for this occurrence is the fact that plant growth will be minimal, if not absent, below this temperature, and since establishment within the rhizosphere is determined by a factor other than rapid growth, there is no requirement for growth below 3-4°C. Remacle (1966) frequently found that the maximum number of organisms within the rhizosphere were counted on a media incubated at a temperature higher than that which gave maximum control soil counts.

The discussion presented earlier concerning acid production from lactose and the amylolytic capabilities applies to the data obtained in the characterization of the 401 isolates. The ability of hydrolyze urea is also less common with the rhizoplane isolates. Urease is also found more frequently in the Pseudomonas than the Achromobacter (von Graevenitz, 1971; Gilardi, 1973). This again suggests a proportionate increase of Achromobacter within the rhizoplane.

The observations of sensitivity to acid pH suggest a slightly more alkaline pH in the rhizosphere (Figure 9). This could be explained by the high water movement required in a draughty site. This would lead to an excess of cations in the rhizosphere, with the associated tendency to a more alkaline pH (Riley and Barber, 1970). In addition, there is a preponderance of nitrate nitrogen in this soil (Appendix 3) that would lead to a more alkaline pH (Riley and Barber, 1971).

The lack of a significantly greater phosphate solubilizing component within the rhizoplane may be accounted for by the frequently observed loss of this ability on extended storage (Sperber 1958). Sperber reported that this occurred more frequently with soil than rhizosphere isolates.

3. Micro-organism - Plant growth interaction

The studies reported in this section are directed toward determining the influence of selected rhizoplane isolates on the growth of lodgepole pine. A prerequisite of such a study is the ability to grow sterile plants at rates equivalent to those achieved in practical operations. In addition, a system with such a capability should of necessity be as simple and inexpensive as feasible.

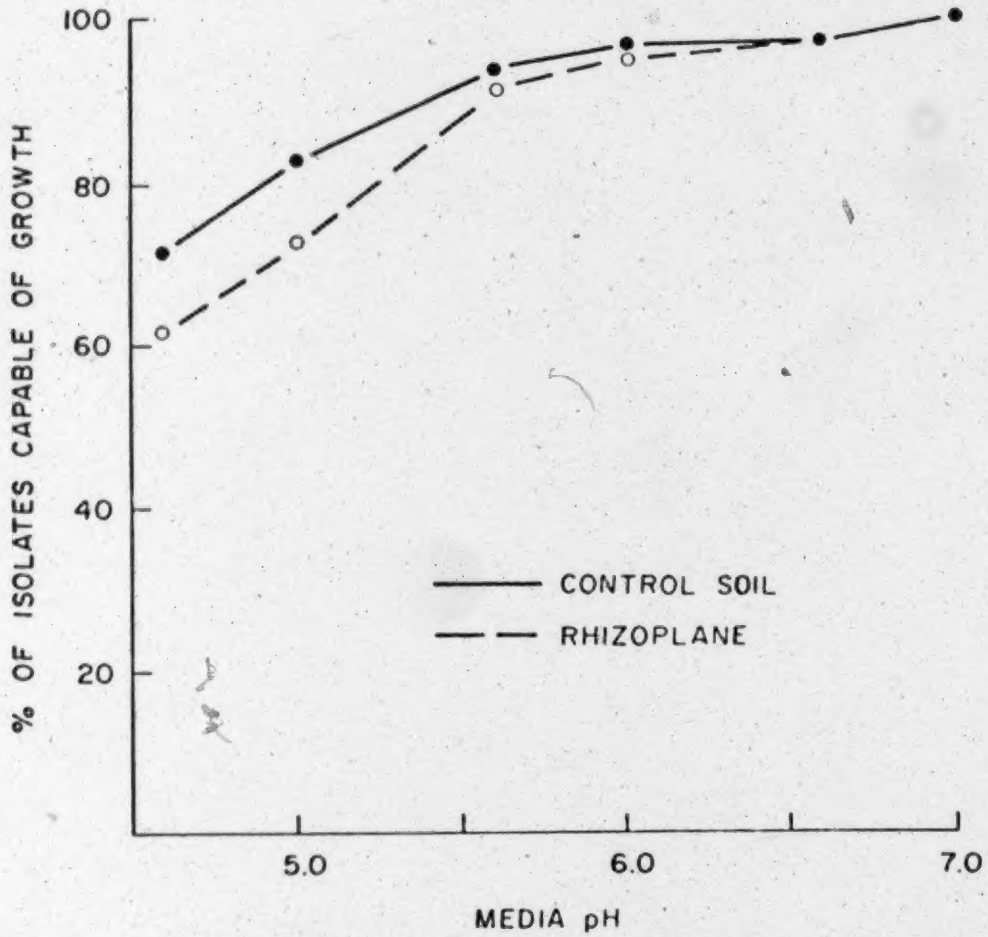

Figure 9. Proportion of rhizoplane and control soil isolates capable of growth on general media adjusted to different pH values.

A. Seed sterilization

A number of compounds have been used to bring about a surface sterilization of the seed coat, with the resultant development of a sterile germinant. The three most commonly used compounds are, however, mercuric chloride, calcium hypochlorite and hydrogen peroxide.

Seeds sterilized by mercuric chloride or calcium hypochlorite must have the residue washed off, while with hydrogen peroxide this is not necessary. Because these seeds tended to be hydrophobic and were difficult to wet in the sterilizing solution, the value of including 70% ethanol in the sterilizing mixture was examined. An initial experiment was conducted to determine the influence of these sterilants on seed germinability, on germinant abnormality or aborted germinations, and on frequency of microbial contamination. The results are presented in Table 13.

From these data, it is apparent that the addition of ethanol to the sterilizing solution dramatically reduces seed germination. It is also apparent that following calcium hypochlorite or mercuric chloride seed sterilization there is a marked increase in the number of abortive or abnormal germinants. On repeated testing, it became apparent that with 30% hydrogen peroxide treatment, only a low number of contaminated seeds remained and only a minimum of abnormal or abortive germinants were produced. In addition, hydrogen peroxide treatment was quick and simple. For these reasons, the 30% treatment was used for routine seed sterilization and the preparation of sterile germinants, which could then be seeded into a

Table 13. Influence of Sterilants on Seed Germination

Treatment	% Contamination	% Germination	% Abortives	% Ungerminated
None	N/A	66	1	33
3% H_2O_2 - 45 min No after rinse	4	63	1	31
30% H_2O_2 - .5 min No after rinse	0	65	3	32
30% H_2O_2 + 70% ethanol (1:1) 2 min with after rinse	12	46	8	34
7% CaOCl 90 min. after rinse	8	61	14	17
.1% $HgCl_2$ 2.5 min after rinse	7	40	28	25
.2% $HgCl_2$ + 70% ethanol (1:1) .5 min with after rinse	0	3	1	96

sterile chamber.

B. Development of the sterile growth system

Initially, an attempt was made to grow plants with the roots in a sterile environment while maintaining the shoot within the normal air flow, as suggested by Stotzky (1962). Such a system is relatively simple and allows monitoring of photosynthesis and respiration as the plant develops. A single seedling was grown in each chamber to reduce the chance of accidental contamination destroying a large portion of any experiment. The chamber initially tried is presented in Plate 5. Two holes were drilled into a #1 rubber stopper to accept 13 mm glass tubes. Each tube was capped

(Bellco Ka-put) for sterilization before a sterile germinant was planted. When the shoot tip of the emerging plant reached the cap, it was removed and the plant "sealed in". As the "sealing in" compound, a mix of liquid paraffin and wax, lanolin and RTV silastic (Stotzky, 1961) were all tried unsuccessfully.

The inability to successfully "seal in" root systems aseptically meant that periodical monitoring of photosynthesis and respiration was impossible as this measurement would place a plant in a non-sterile environment.

Because "sealing in" was no longer feasible, the rubber stopper was removed and the sterile chember was further modified (Plate 6). Aeration in this system is by diffusion. Initial growth was excellent but gradually slowed down and, after 9 weeks, the plants had set bud. While growth was much improved, it was inadequate when compared with operationally grown seedlings.

A number of variations of the above apparatus and conditions were tested in order to improve the growth rate of sterile seedlings.

a. Soil mix and nutrient solution: Sterilized seed were grown in 8 ounce jars containing vermiculite-sand mix in a 2:1 or 1:2 ratio (Appendix 4). Watering and nutrient application over the 11 weeks from seed sterilization to harvest were applied according to the growth schedule outlined in Appendix 6. In all cases, the 2:1 vermiculite-sand mix produced a significantly larger plant in the 77-day growing period (Table 14). With Hocking's nutrient solution, the difference was significant at the 95% level and, with

Plate 5. System initially used to grow seedling with aseptic root system while maintaining stem in normal air stream.

Plate 6. System for growing sterile plants with air provided by diffusion.

the commercial preparation, at the 99% level. The improved growth may be explained by the increased moisture present, or improved air exchange that results from the larger pore size of this mix. As a result, the 2:1 mix was used in all subsequent experiments.

Table 14. Influence of Soil Mix and Nutrient Source on Seedling Growth

	Oven-Dry Weight of Plants in (mg)	
	Hocking Solution	Commercial Fertilizer
2:1 Vermiculite and sand	305	297
1:2 Vermiculite and sand	220*	224**

* Significantly different 95% level

** Significantly different 99% level

The influence of two nutrient solutions is also compared in Table 14. There was no significant difference in total plant weight. Because Hocking's solution provided a known concentration of micronutrients, this was the accepted choice for further studies.

b. Aeration: One possible explanation for the poor growth within the sterile chamber was an inadequate CO_2 supply to meet plant demand. This was checked by growing plants in the chamber shown in Plate 6. Plants, which were to act as open controls, were started in the closed sterile system and, at 4 weeks from seeding, the upper half of the chamber was opened. It is apparent (Table 15) that the major problem associated with poor plant growth in the sterile chamber is a lack of adequate CO_2 supply. Visual examinations made during the growth period studied indicate that differences in growth are apparent by 7 weeks when several seedlings are grown in the chamber, and by 8 weeks when a single seedling is grown. Many seedlings also set bud in the 9th week.

Table 15. Influence of Sterile System on Seedling Growth Over 77-Days

	Total Plant Oven Dry Weight (mg)	
	Open to Air	Sterile System
2:1 Vermiculite and sand (Hocking)	298	67
1:2 Vermiculute and sand (Hocking)	220	61
2:1 Vermiculite and sand (Commercial fertilizer)	273	58
2:1 Vermiculite and sand (Commercial fertilizer)	212	66

If aeration is to be provided by diffusion, it would appear that 6 weeks is the maximum time over which near-normal growth rates might be expected. It was thus obvious that for extended growth periods, the chamber must be supplied with a constant flow of sterile air. The pressurized air flow system used in the completion of this study is displayed in Plate 7 and Appendix 8. This apparatus was further modified and improved to reduce contamination opportunity and time required for watering (Plate 8 and Appendix 8).

C. Influence of selected inoculations on seedling characteristics

The final objective of any rhizosphere work would be to use this information to manipulate rhizosphere microflora to the plants' "benefit".

The results presented in Table 16 are the first attempt at

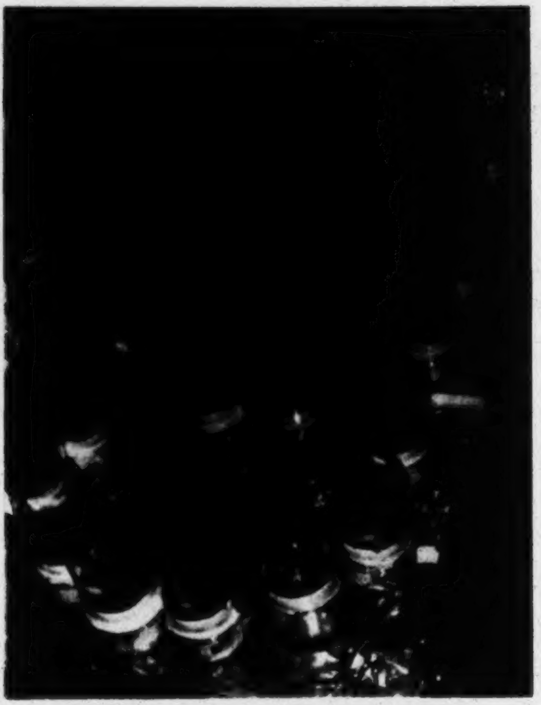

Plate 7. System for growing sterile plants with pressurized sterile air flow.

Plate 8. Sterile plant growth system with pressurized sterile air stream and gravity feed nutrient reservoir attached.

Table 16. Plant Growth* Characteristics As Influenced By Microbial Inoculation

Treatment (inoculum)	Organism #s/gm rhizosphere soil	Shoot Height (cm)	Root Wt (mg)	% of Control	Shoot Wt (mg)	% of Control	S/R	% of Control	Total Wt (mg)
Sterile	None	3.6	23.4	100	38.8	100	1.82	100	62.2 ab**
Mix***	62×10^7	3.5	21.8	93	34.2	88	1.66	90	56.0b
14b	44×10^6	3.6	25.8	110	37.4	96	1.61	102	63.2ab
47c	39×10^4	3.6	27.5	117	38.8	100	1.59	107	66.3a

* Plant growth 102 days after seeding (Sterilization)

** Values followed by the same letter are not significantly different at the 95% level.

*** See Appendix 9 for characterization and basis of selection for pure culture inoculum.

establishing specific organisms within the rhizosphere and observing their effect. These plants were grown with air supplied by diffusion (Plate 6) and as a result were very small at harvest date. Only in the case of total weight are any of the differences significant. It is interesting to note the increased root growth induced by the inoculation with organisms 14b and 47c that results in a decrease of shoot/root ratio.

All differences in the organism counts (Table 16) from the treatments are significant at the 99% level. There are no relationships between observed plant growth and organism establishment within the rhizosphere.

Because of the poor growth of plants in this original interaction study, the work was repeated but plants were grown in the aseptic chambers with a pressurized air flow (Plate 7). Samplings were made at 65 and 101 days from seed sterilization (Table 17). The difference in total weight (65 days) between the sterile control and inoculum 47c was significant at the 95% level, as it was in the first test. This was not so with 14b and mixed culture inoculum, although they were only slightly smaller than the 47c plants. The slightly larger S/R ratio for the inoculated plants is an indication of more rapid growth. It is during this stage, under ideal conditions, that the plant produces a great deal of top growth (Van Eerden, personal comm.).

By 105 days, the trends changed and the mixed culture inoculated plants were smaller than any other treatment. This was identical to the results for the initial test (Table 16). Again, the inoculated

Table 17. Plant Growth On Inorganic Nitrogen As Influenced By Microbial Inoculation

Inoculum	Root Wt (mg)	Shoot Wt (mg)	Total Wt (mg)	S/R
		Growth For 65 Days From Seed Sterilization		
Sterile	23.7	57.9	81.6 a*	2.51
Mix	26.8	76.2	103.0 ab	2.87
14b	28.6	75.0	103.6 ab	2.63
47c	27.2	78.9	106.3 b	2.94
		Growth For 101 Days From Seed Sterilization		
Sterile	94	238	332	2.53
Mix	73	177	251	2.42
14b	144	241	385	1.67
47c	126	223	349	1.77

* letters followed by different numbers are significantly different at the 95% level.

plants had the lower shoot/root ratio. While not significant, the reproducibility of the observations was at least gratifying. The change between observations made at 65 and 105 days emphasized the importance of making comparisons at similar points in the growth cycle. Swan (1969) has suggested that for real differences in growth rate of jack pine seedlings to become evident, the maximum growth period feasible should be used for assessment. He used a 26-week (182-day) growing period. That period is unrealistic for this study, because in the operational growth of container-grown reforestation stock, the growth period is 18-20 weeks (126-140 days). Any changes in plant characteristics must therefore be induced by the end of that time period.

As stated earlier, one of the requirements of a system for the growth of aseptic plants was a growth rate equal to those achieved operationally. Endean (1971) has reported maximum plant weights of 270 mg for lodgepole pine grown 108 days under light, temperature and nutrient conditions similar to those used in this experiment. The major difference was a peat-soil mix and the use of a "Styro 2" container. Comparison of Endean's data, with that in Table 17, indicates equal or better growth rates within the sterile chamber. Comparisons with the hypothetical growth curve (Appendix 6) drawn from published information are equally favorable.

When three-quarters of the nitrogen in Hocking solution was replaced with an organic nitrogen source, the effect was to reduce nitrogen availability and make the plant somewhat dependent on the associated micro-organisms for the needed nitrogen. At the age of 65 days, the organic nitrogen-grown plants (Table 18) were not significantly smaller than the inorganically grown plants (Table 17), although that trend is suggested by the growth rates of the inoculated plants. That these plants are not doing as well is also suggested by the lower S/R ratio of the organic N growth plant. Plants grown under lower nitrogen availability have a lower S/R ratio (Swan, 1969; Kinghorn, personal comm.) and a reduced shoot height growth. In this experiment, the height growth at 65 days was 60 mm and 71 mm, respectively, for the organic and inorganic

Table 18. Plant Growth on Organic Nitrogen as Influenced by Microbial Inoculation.

Inoculum	Root Wt (mg)	Shoot Wt (mg)	Total Wt (mg)	S/R
		Growth Period of 65 Days from Seeding		
Sterile	25.6	48.3	73.9	1.91
Mix	26.3	61.3	87.7	2.33
14b	29.8	57.9	87.8	1.95
47c	29.8	54.8	84.6	1.84
		Growth Period of 101 Days from Seeding		
Sterile	115	105	220	.94 a*
Mix	103	152	255	1.50 b
14b	119	134	253	1.13 ab
47c	83	125	208	1.62 b

* Numbers followed by the same letter are not significantly different at the 95% level.

nitrogen-grown plant. Similar observations were evident at 101 days, where the trend was to a smaller plant. None of these differences are significant. The data from the 101-day sampling indicate a significantly different shoot-to-root ratio brought about by the presence of the mix and 47c inoculum. Since this is an increase in shoot-to-root ratio, it is probably the result of an increased nitrogen availability. In the organism characterizations, both 47c and 14b were proteolytic positive, while only 47c was positive for ammonium production from casein.

Bacterial counts within the rhizosphere soil at the end of the 65-day growth period for plants grown on an inorganic N source (Table 19) are not significantly different from those after 102 days' growth (Table 16). It is not surprising that the count for the soil mix inoculum are significantly larger on the organic nitrogen, since the casein will act as an additional energy source. The rhizoplane inoculum, 47c, appears to be uninfluenced by the external energy supply and is restricted in activity by the particular root organism association.

Table 19. Organism Counts Within 65-Day-Old Seedling Rhizosphere Soil.

	Inorganic N	Organic N
Mix	72×10^6	88×10^7
47c	82×10^4	82×10^4
14b	32×10^5	14×10^6

V. Conclusions

Data presented in this thesis have provided some information on the role of the bacterial component in lodgepole pine rhizosphere soil and have demonstrated a number of differences from that of the non-root-influenced soil. As in most similar studies, the number of bacteria increases when soil is subjected to root influence. The streptomycetes increased to a lesser extent than the bacterial component. In the mature tree-root studies, the rhizosphere effect increased with depth and is primarily associated with feeding roots. The magnitude of the stimulation within the rhizoplane is striking when all data are expressed and compared on a surface area basis (number of organisms/100 sq cm).

If the total number of organisms is accepted as a rough indicator of microbial activity, a majority of all soil activity takes place right on the root surface.

The preliminary study indicated that the almost universally reported stimulation of gram-negative organisms within the rhizosphere is not present in the lodgepole pine rhizosphere soil. This may be a result of a high proportion of gram-negatives within the control soil. In further contrast to other reports, rhizoplane organisms do not demonstrate as rapid a growth rate as the non-root-influenced soil isolates. They do, however, respond in greater proportions to amino acid nutrient supplementation than do the control organisms.

The rhizosphere isolates have consistently demonstrated an increased ability to bring about phosphate solubilization and may be of importance to the phosphorus status of the plant.

Proteolytic and amylolytic organisms were found to be suppressed within the rhizosphere. The suppression of proteolytic organisms is in contrast to many other observations and would indicate a reduced nitrogen turnover within the rhizosphere. In addition, ammonifiers were also reduced within the rhizosphere. These observations, combined with the reduced frequency of acid production from lactose and of urea hydrolysis within the rhizoplane microflora, suggest a proportional increase in the Achromobacter within the rhizoplane.

Chitinolytic organisms were suppressed within the rhizosphere of the mature tree, while being stimulated within the rhizosphere of the young seedling or sapling. With this exception, the observations based on a very limited number of characteristics suggest that the rhizosphere flora of older and younger trees are similar.

The incorporation of environmental tests indicated that the rhizoplane flora was slightly more sensitive to acid pH. This would suggest a more alkaline pH in the rhizosphere. The rhizoplane isolates were also less capable of growth at 3-4°C.

A sterile growth chamber has been developed that allows growth rates, under sterile conditions, equivalent to those achieved in operational practices. Studies of micro-organism/plant interactions with a limited number of organisms have indicated that these organisms can have a definite effect on the plant growth rate and the distribution of this growth. The potential for controlling seedling characteristics is thus demonstrated, but the means of controlling the inoculum in a non-sterile situation is unavailable.

These results demonstrate the need for pursuing the study of the rhizosphere microflora and developing a mechanism for controlling the organisms associated with seedlings in the initial phase of growth.

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* An asterisk preceding a citation indicates that only the abstract of the paper was available and read.

APPENDIX 1

Microbiological Media

A. Isolation Media

1. Bacteria and Actinomycetes

a) Stevenson Yeast Extract Agar

(Cook, personal comm.; Lochhead and Chase, 1943; Corke and Chase, 1955).

Ingredients	grams/liter
Dextrose	1
K_2HPO_4	1
$(NH_4)_2SO_4$	.5
$MgSO_4 \cdot 7H_2O$	.2
$CaCl_2$	.1
NaCl	.1
$FeCl_3$	.01
Yeast Extract	1
Agar	15

Actidione is added to the sterilized above medium when at 50°C to give a final conc. of 80 nanogm/ml.

b) Plate Count Agar (Difco Laboratoirie Inc.)

Ingredients	grams/liter
Tryptone	5
Yeast Extract	2.5
Dextrose	1
Agar	15

c) Soil bacteria mix (Dangerfield, unpublished data)

Ingredients	grams/liter
K_2HPO_4	1
glucose	1

Ingredients	grams/liter
$(NH_4)_2SO_4$	.5
$MgSO_4 \cdot 7H_2O$	.2
$CaCl_2$	.1
NaCl	.1
$FeCl_3$	.01
Avicel (TG 104)	1
Starch	1
Casein	.25

This medium is adjusted to pH 6.8.

Filter-sterilized Actidione is added after autoclaving to give a final concentration of 80 nanogm/ml.

2. Fungi

a) Peptone-Dextrose-Rose Bengal Agar (Martin, 1950)

Ingredients	grams/liter
Agar	20
KH_2PO_4	1
$MgSO_4.7H_2O$	0.5
Peptone	5
Dextrose	10

Rose Bengal (1% Solution) is added, 3.3 ml/liter after remainder of medium is boiling.

Streptomycin is added, 30.0 mg/liter after cooling autoclaved medium to 45°C.

B. Storage Media

1. Bacteria

a) General medium (Dangerfield, unpublished data)

Ingredients	grams/liter
Dextrose	1
Tryptone	5
Yeast Extract	2.5

APPENDIX 1

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$CaCl_2$	.1
NaCl	.1
$FeCl_3$	.01
Yeast Extract	1
Agar	15

Actidione is added to the sterilized above medium when at 50°C to give a final conc. of 80 nanogm/ml.

b) Plate Count Agar (Difco Laboratoirie Inc.)

Ingredients	grams/liter
Tryptone	5
Yeast Extract	2.5
Dextrose	1
Agar	15

c) Soil bacteria mix (Dangerfield, unpublished data)

Ingredients	grams/liter
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Streptomycin is added, 30.0 mg/liter after cooling autoclaved medium to 45°C.

B. Storage Media

1. Bacteria

a) General medium (Dangerfield, unpublished data)

Ingredients	grams/liter
Dextrose	1
Tryptone	5
Yeast Extract	2.5

Ingredients	grams/liter
Vitamin Free Casamino Acid	2.0
Citric Acid	.05

This medium is prepared as a broth, semi-solid agar (0.5% agar) or as solid agar (1.5% agar).

2. Fungi

a) Malt Extract Agar (Difco Laboratorie Inc.)

Ingredients	grams/liter
Agar	12.75
Maltose	2.75
Dextrin	2.35
Glycerol	0.75
Peptone	15

C. Organism Characterization

1. Fungi

a) Czapek Solution Agar (Difco Laboratories Inc.)

Ingredients	grams/liter
Agar	15
$NaNO_3$	2
K_2HPO_4	1
$MgSO_4 \cdot 7H_2O$	1
KCl	5
Saccharose	30
$FeSO_4$	.01

b) Potato Dextrose Agar (Difco Laboratories Inc.)

Ingredients	grams/liter
Potatoes, Infusion from	200
Dextrose	20
Agar	15

2. Bacteria

a) Starch hydrolysis (Sundman, 1970)

General medium solid agar plus 0.2% soluble starch. 5 ml of this medium is layered over 15 ml of water agar. Colonies are incubated for 7 days at 20°C.

A zone of clearing around the colony after flooding with 1/10 strength Lugol's iodine was scored positive for starch hydrolysis.

Lugol's Iodine	
Iodine	5 g
Potassium Iodine	10 g
Distilled Water	100 ml

b) Chitin hydrolysis (Gray and Bell, 1963)

Ingredients	grams/liter
Hydrolyzed Chitin	5
K_2HPO_4	.3
$MgSO_4$	.3
$CaCl_2$	.1
$FeSO_4$	.01
Glucosamine HCl	.05
Vitamin Free Casamino Acid	.2
Yeast Extract	.2
Agar	15

The pH of this media is adjusted to 7.0 before autoclaving. This medium is layered 5 ml over 15 ml of water agar.

A zone of clearing around a colony in 14 days at 20°C is scored positive for chitin hydrolysis.

PREPARATION OF HYDROLYZED CHITIN: Dissolve powdered Chitin 1:10 in concentrated HCl at 50°C. Filter through glass wool and reprecipitate by dilution in distilled water. Wash precipitate three times, neutralize with NaOH and rewash.

c) Phosphorus solubilization (Katznelson and Bose, 1959)

General medium solid agar plus 50 ml of a sterile 10% K_2HPO_4 and 100 ml of a sterile 10% $CaCl_2$ per liter. These solutions are mixed at 45°C before 5 ml of the completed medium is layered over 15 ml water agar.

A zone of clearing around a colony in 14 days at 20°C is scored positive for phosphorus solubilization.

Media for the Preliminary Study

Ingredients	
Glucose	1 g
Agar	15 g
Soil Extract (Lochhead)	1000 ml

After sterilization and cooling to 45°C mix in the following sterile solutions.

K_2HPO_4	(10%)	15 ml
$CaCl_2$	(10%)	30 ml

Adjust to pH 7.0 with Sterile NaOH.

d) Casein hydrolysis (Cook, personal comm.)

Ingredients	grams/liter
Powdered Skim Milk	15
Yeast Extract	.5
Vitamin Free Casamino Acid	.2
Citric Acid	.05
Agar	15

This medium is layered 5 ml over 15 ml of water agar.

A zone of clearing around a colony in 7 days at 20°C is scored positive and indicative of casein hydrolysis.

e) Lipolysis of Tween 80 (Sierra, 1957)

General medium solid agar plus Tween 80 which is sterilized separately and added at a rate of 5 ml/liter when at 45°C.

An opaque halo around a colony following 7 days incubation at 20°C is scored positive and indicative of lipolytic activity.

f) Phosphatase (Cowan and Steel, 1965. Page 162)

General medium solid agar plus 10 ml/liter of a 1% phenolpthalein phosphate solution which has been filter-sterilized. These solutions are mixed when at 45°C and just before plates are poured.

Inoculated plates are incubated 7 days at 20°C before a drop of conc NH_4OH is placed in the top of the inverted petri dish lid. Colonies becoming a bright pink are scored positive and indicative of phosphatase activity.

g) Oxidase (Blair et al., 1970. Page 679)

General medium solid agar was used to grow up cultures for 48 hr at 20°C. At this time one of two tests was performed.

1) A couple of drops of a 1% solution of tetramyethyl-p-phenylenediamine dehydrochloride is added to the colony surface. Colonies developing a dark purple color in 60 sec are scored positive.

2) Culture scrapings using a platinum loop are transferred to a filter paper dampened with the reagent. Colonies developing a purple color in 60 sec are scored positive for oxidase activity.

h) Catalase (Blair et al., 1970. Page 675)

General medium broth buffered at pH 7.0.

Cultures drop inoculated and incubated for 7 days at 20°C were treated with ½ ml of 3% H_2O_2. Gas production is scored as positive and is indicative of catalase formation.

i) Urease (Difco Laboratories Inc.)

Ingredients	grams/liter
Yeast extract	.1
Na_2HPO_4	9.5
KH_2PO_4	9.1
Urea	20
Bacto-phenol red	.01

This medium is filter-sterilized. Drop inoculated cultures incubated at 20°C are examined daily for 7 days. A red coloration is scored positive for urease.

j) Basal medium (Lochhead and Chase, 1943)

Ingredients	grams/liter
Glucose	1.0
K_2HPO_4	1.0
$(NH_4)_2SO_4$	.5
$MgSO_4$	.2
$CaCl_2$	.1
NaCl	.1
$FeCl_3$	.01

k) Amino acid medium (Stevenson and Rouatt, 1953)

Basal medium plus 0.4% of vitamin free casamino acid (Difco)

Typical Analysis of Bacto-Casamino Acid (vitamin free)

Ash	39.0%
Total Nitrogen	8.0%
Amino Nitrogen	6.5%

Amino Acids (in percentages)

Arginine	2.0

Aspartic Acid	5.0
Glutamic Acid	15.0
Glycine	1.0
Histidine	1.5
Isoleucine	4.0
Leucine	5.0
Lysine Methionine	5.0
Methionine	1.0
Phenylalanine	2.0
Threonine	2.0
Tyrosine	1.0
Valine	4.0

1) Yeast - amino acid medium (Dangerfield, unpublished data)

Amino acid medium plus 0.1% of yeast extract (Difco)

Typical Analysis of Bacto - Yeast Extract

Ash	10%
Total Nitrogen	9.5%
Amino Nitrogen	7%
Amino Acids (in percentages)	
Arginine	1
Aspartic Acid	5
Glutamic Acid	6.5
Glycine	2.5
Histidine	1
Isoleucine	3
Leucine	3.5
Lysine	4
Methionine	1
Phenylalanine	2
Threonine	3.5
Tyrosine	0.5
Valine	3.5

Vitamin Factors (micrograms per gram)	
Pyridoxine	20
Biotin	1
Thiamine	3
Nicotinic Acid	280
Riboflavin	20

m) Yeast extract medium (Lochhead and Chase, 1943)

Basal medium plus 0.1% yeast extract

n) Yeast - Soil extract (Lochhead and Chase, 1943)

Yeast-amino acid medium in which one quarter of the distilled water was replaced with soil extract. The soil extract was prepared by autoclaving 1000 g of soil in 1000 ml of water for 20 minutes (Lochhead, 1940). This was allowed to cool overnight before filtering and making the extract volume to 1000 ml.

o) Ammonification (Cook, personal comm.)

Ingredients	grams/liter
Nutrient broth (Difco)	16
Casein (Difco)	5

Incubate with inoculation for 7 days at 25°C and test for free NH_4^+ with Nessler's reagent.

Nessler's Reagent (Cowan and Steel, 1970. Page 148)

Dissolve 5 g potassium iodide in 5 ml freshly distilled water. Add cold saturated mercuric chloride solution until a slight precipitate permanently remains after thorough shaking. Add 40 ml 9N NaOH. Dilute to 100 ml with distilled water and allowed to stand for 24 hr.

Notes. The water used in its preparation must be ammonia-free. Allow the reagent to settle before use. Protect from light.

Yellow precipitate indicates the presence of free ammonia

p) Nitrate reduction

Ingredients	grams/liter
KNO_3	3.0
Bacto-Beef Ext	1.5
Bacto-Yeast Ext	1.5
Bacto-Peptone	5
Bacto-Dextrose	1
Sodium Chloride	3.5
Dipotassium Phosphate	3.68
Mono Potassium Phosphate	1.32

After growth for 5 days, the culture is tested for the presence of nitrite with Griess reagent. A pink color indicates the nitrite ion and thus nitrate reduction. In the absence of nitrite, the sample must be checked to see that all nitrate has not been removed and thus the lack of nitrite can be explained by the lack of nitrate substrate. This is carried out by adding a few grains of a powdered metal mixture to the negative reaction solution. Any nitrate present is reduced to nitrite and a pink color develops. Nitrate presence was also checked in some cases by mixing 2 drops of culture with 1 drop each of concentrated H_2SO_4 and diphenylamine. A deep blue indicates nitrate or nitrite.

Griess reagent (Cowan and Steel, 1970. Page 148)

Solution A

Sulfanilic acid	8 grams
Acetic acid (5N)	1 liter

Solution B

Napthylamine	5 g

Acetic acid (5N)	1 liter

The 5N acetic acid consists of 1 part glacial acetic acid to 2.5 parts distilled water. Equal parts of A and B are mixed just prior to use.

Powdered metal mixture (Alexander and Clark, 1965)

Zinc powder	1 gram
MnO_2 powder	1 gram
Copper powder	0.1 gram

Diphenylamine Reagent

1) Dissolve 0.7 g of diphenylamine in a mixture of 60 ml of conc sulfuric acid and 28.8 ml of distilled water.
2) Cool this mixture and add slowly 11.3 ml of conc HCl. Let stand overnight.
3) After standing overnight, some of the bases will separate, showing that reagent is saturated.

q) Methylene-blue reduction (Katznelson and Rouatt, 1957a)

General medium broth plus methylene blue added to a concentration of 5 ppm. Medium is tubed, sterilized and drop-inoculated. Cultures are examined daily for 7 days while incubated at 20°C. Clearing of medium is scored as positive for methylene-blue reduction

r) Growth at different pH (Munro, 1970)

Solution A	0.1 M Citric Acid
Solution B	0.2 M Na_2HPO_4

Solution A and B are mixed in the volume indicated in the attached table for the desired pH. These are then diluted to 1 liter and the dry components of the General Medium added.

pH	ml A	ml B
4.0	307	193
4.6	267	223
5.0	243	257
5.6	210	290
6.0	179	321
6.6	136	364
7.0	65	436

Cultures are drop-inoculated in this broth and incubated at 20°C for 7 days. Visible turbidity is taken as evidence of growth and is scored positive.

s) Growth at different temperatures (Sundman, 1970)

Organisms are inoculated to General Medium and incubated at the desired temperature prior to examination for turbidity which was taken as evidence for growth and scored positive.

3-4°C	incubate for 21 days
37°C	incubate for 7 days

t) Oxidative-fermentative carbohydrate metabolism (Board and Holding, 1960)

Ingredients	grams/liter
$NH_4H_2PO_4$	.5
K_2HPO_4	.5
Yeast Extract	.5
Agar	5.0
Bromothymol Blue (1% solution at pH 7.2)	3 ml

This medium was sterilized before filter-sterilized carbohydrate was added to give a final concentration of 0.5% W/V. This medium was then aseptically dispensed into 13 x 100 mm tubes in

5 ml lots.

Cultures are "stab"-inoculated and incubated at 20°C. All tubes are examined daily for 7 days and then at 14 and 21 days.

BLUE	ALKALINE pH
YELLOW	ACIDIC pH

A yellow color throughout the tube is indicative of acid formation and a fermentative type of metabolism.

u) Bacterial gram staining (Society of American Bacteriologists, 1957. Page 16).

BACTERIAL GRAM STAINING -

Hucker Modification

Ammonium Oxalate Crystal Violet (Hucker's)

Solution A		Solution B	
Crystal violet (90% dye content)	.2 g	Ammonium oxalate ...	0.8 g
Ethyl alcohol (95%)	20 ml	Distilled water	80 ml

Mix solutions A and B

Gram's Modification of Lugol's Solution

Iodine	1 g
KI	2 g
Distilled water	300 ml

Counterstain

Safranin O (2.5% solution in 95% ethyl alcohol)	10 ml
Distilled water	100 ml

Staining schedule:

1) Stain smears 1 min with ammonium oxalate crystal violet. This formula has sometimes been found to give too intense staining, so that certain gram-negative organisms (e.g. the gonococcus) do not properly decolorize. If this trouble is encountered, it may be

avoided by using less crystal violet.

2) Wash in tap water for not more than 2 sec.

3) Immerse 1 min in iodine solution.

4) Wash in tap water, and blot dry.

5) Decolorize 30 sec with gentle agitation, in 95 per cent ethyl alcohol. Blot dry.

6) Counterstain 10 sec in the above safranin solution.

7) Wash in tap water.

8) Dry, and examine.

Results: Gram-positive organisms, blue; gram-negative organisms, red.

APPENDIX 2

Surface Area of Small Roots

Root volume, as determined by displacement, is subject to error caused by penetration of the liquid into the root and by entrapment of small air bubbles on the root surface. Penetration of the liquid of high viscosity. Trapping of air bubbles, which increases the determined volume, can be reduced by using a liquid of low surface tension. Since root material has a specific gravity of less than one, a liquid with a low specific gravity would help to reduce errors caused by converting weights to volume. For these reasons, ethanol was selected as the best liquid to use for determination of root volume by displacement.

Ethanol

Specific gravity	0.791 g/cm^3
Viscosity	1.003 cp
Surface Tension (20°C)	22.75 dyne/cm

1. Typical Determination of Root Surface Area

Root length was measured to the nearest millimeter. The root segments were then placed in a wire mesh basket, which weighed 3.9519 g and 3.5573 g, suspended in air and ethanol, respectively. The weight of basket and roots was then determined while suspended in air and ethanol.

Weight in Air	5.6501 g
Weight in Ethanol	2.9154
Volume Equivalent Wt (Root + Basket)	2.7347
Volume Equivalent Wt (Basket)	.3946
Volume Equivalent Wt (Root)	2.3401

Volume of the root = Inverse of specific gravity times the volume equivalent weight of the root.

Therefore

$$\text{Volume} = 2.3401 \text{ g} \times \frac{1000 \text{ mm}^3}{0.791 \text{ g}}$$

$$= 2958 \text{ mm}^3$$

If we assume that the root segment is a cylinder then

$$V = \pi r^2 h$$

$$\text{or } r^2 = \frac{V}{\pi h}$$

Substituting for h = 736 mm we have

$$r^2 = \frac{2958 \text{ mm}^3}{\frac{22}{7} \times 736 \text{ mm}}$$

$$= 1.28 \text{ mm}^2$$

$$r = 1.13 \text{ millimeters}$$

Since the surface area of a cylinder is defined by the formula $2 \pi rh$, we can calculate the surface of the root.

$$\text{S.A.} = 2 \times \frac{22}{7} \times 0.113 \times 73.6 \text{ cm}$$

$$= 52.3 \text{ sq cm}$$

2. Calculation of total counts/gm from original dilution data

Example: Total weight of sample added to 90 ml of sterile water was 10.6 g. Oven-dry weight of material filtered out as 4.62 g.

Initial dilution factor $\frac{100.6}{4.6} = 21.8$

Counts were 67.3 colonies (average) on the listed 10^4 dilutions.

Since the initial dilution was approximately 22 and not 10, the calculation is made as outlined below.

$$22 \times 67.3 \times 10^3 = 147 \times 10^4 \text{ bacteria/g}$$

3. Conversion of data to counts per 100 sq cm

Counts from all samples were initially calculated on a per gram basis. This required that all surface area information be converted to a per gram constant for each sample.

a) Rhizoplane

Example. (total counts/g) = 147×10^4

In this case, 4.62 g of the original material placed in the dilution bottle had a surface area of 106.7 sq cm.

$$\frac{106.7 \text{ sq cm}}{4.62 \text{ g}} = 23.1 \text{ sq cm/g}$$

Converting to total counts/100 sq cm

$$= \left(\frac{147 \times 10^4 \text{ bacteria}}{\text{g}} \times \frac{\text{g}}{23.1 \text{ sq cm}}\right) 100$$

$$= 637 \times 10^4 \text{ bacteria/100 sq cm}$$

b) Rhizosphere and Control Soil

The surface area of the soil is calculated in sq meters/g

1 sq meter = 10,000 sq centimeters

Since the conversion is to counts/100 sq cm, we proceed as in the example below.

TC_1 Count = 49×10^4 bacteria/g

Surface Area = 14.9 sq meters/g

Converting we have

$$= \frac{49 \times 10^4 \text{ bacteria}}{\text{g}} \times \frac{\text{g}}{14.9 \text{ sq meters}}$$

$$= 3.29 \times 10^4 \text{ bacteria/sq meter}$$

$$= 3.29 \times 10^4 \text{ bacteria}/10^4 \text{ sq centimeters}$$

$$= 3.29 \times 10^2 \text{ bacteria}/10^2 \text{ sq centimeters}$$

$$= 329 \text{ bacteria/100 sq centimeters}$$

APPENDIX 3

Soil Analyses

Sample	pH	Extractable Nitrogen		P_2O_5	% of Total Exchangeable Cations				Total C.E.C. me./100g sum of Ex. Cat.	Surface Area
		NH_4^+ ppm	NO_3^- ppm	ppm	H^{+*}	Na^+ and K^+	Ca^{++}	Mg^{++}		m^2/g
					Young Tree Site					
#1	5.6	7.4	16	15	19	4	56	21	7.9	78.5
#2	6.4	7.9	16	19	14	3	69	14	5.8	73.8
#3	5.3	6.8	14	14	41	6	39	13	12.7	94.3
#4	5.3	8.5	12	22	27	3	52	18	12.4	90.2
					Mature Tree Site					
Ae	3.4	0	**	4	31	3	60	6	6.5	14.4
Bf	3.9	.5	**	14	58	4	28	10	5.3	34.8
BC_1	4.3	**	**	8	31	7	48	14	4.2	15.7
BC_2	4.7	.1	**	9	14	4	70	12	5.5	14.6

* Exchange acidity

** Trace

APPENDIX 4

Soil Mix

Two:one V/V	Vermiculite and sand mix
27 g	Vermiculite
91 g	Sand
0.7 ml	1 M HCl
115 ml	distilled H_2O
One:two V/V	Vermiculite and sand mix
13.5 g	Vermiculite
182 g	Sand
0.7 ml	1 M HCl
75 ml	distilled H_2O

Note: 13.5 grams of vermiculite and 91 grams of sand occupy equal volumes.

APPENDIX 5

Nutrient Feed Solutions

Macro-Elemental Composition

N: 112, P: 31, K: 156, Mg: 48, Ca: 80 ppm

1. Inorganic Nitrogen Solution (Hocking, 1971)

Stock solution 1. Use 4 ml per liter of feed solution

Chemical	Quantity
Distilled H_2O	2 litres
H_2SO_4 (1%)	5 ml
NH_4Cl	107.0 g
K_2SO_4	87.0 g
K_2HPO_4	87.0 g
H_3BO_3	1.125 g
MoO_3	0.007 g

Stock solution 2. Use 4 ml per liter of feed solution

Chemical	Quantity
Distilled H_2O	2 litres
$Ca(NO_3)_2 \cdot 4H_2O$	236.0 g
$MgCl_2 \cdot 6H_2O$	203.0 g
$MnCl_2$	0.5 g
$ZnCl_2$	0.05 g
$CuCl_2 \cdot 2H_2O$	0.025 g
$FeCl_3 \cdot 6H_2O$	1.35 g

2. Organic Nitrogen Solution

Stock solution 1. Use 4 ml per liter of feed solution

Chemical	Quantity
Distilled H_2O	2 litres
H_2SO_4 (1%)	5 ml
K_2SO_4	87.0 g
K_2HPO_4	87.0 g
H_3BO_3	1.125 g
MoO_3	0.007 g

Stock solution 2. Use 4 ml per liter of feed solution

Chemical	Quantity
Distilled H_2O	2 litres
$MgCl_2 \cdot 6H_2O$	203.0 g
$MnCl_2$	0.5 g
$ZnCl_2$	0.05 g
$CuCl_2 \cdot 2H_2O$	0.025 g
Ferric chloride $FeCl_3 \cdot 6H_2O$	1.35 g

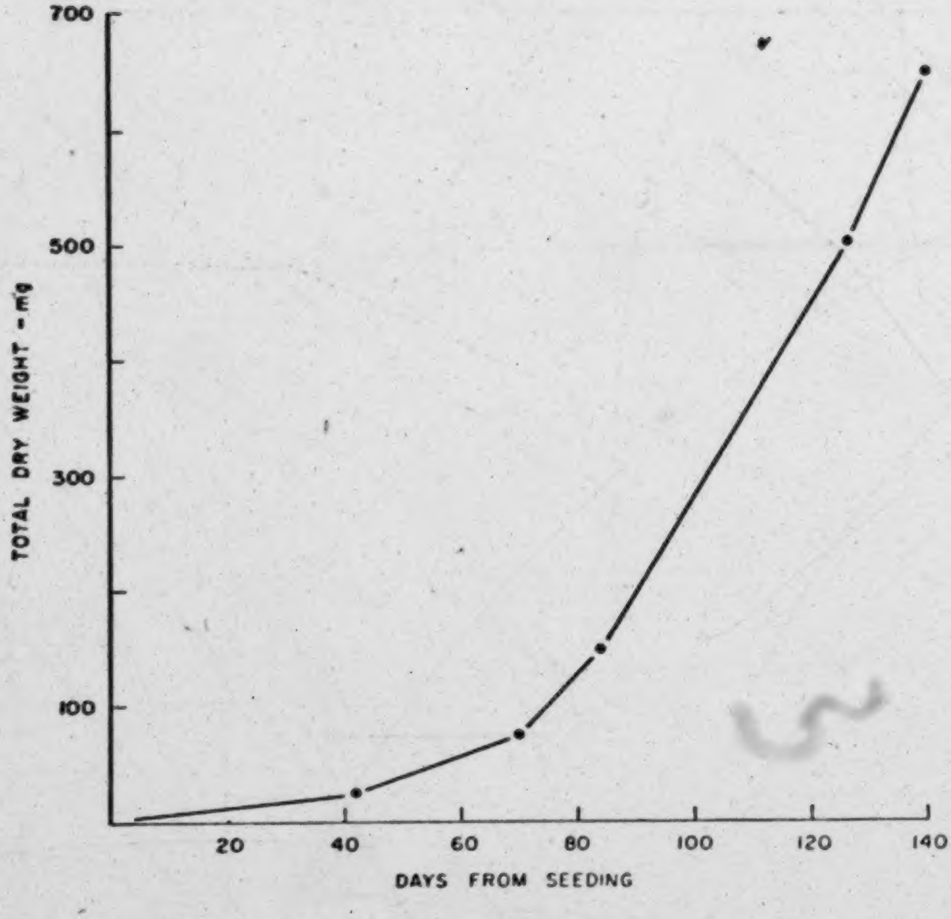
Stock solution 3. Use 4 ml per liter of feed solution

Chemical	Quantity
Distilled H_2O	2 litres
$Ca(NO_3)_2 \cdot 4H_2O$	59.0 g
NH_4Cl	26.75 g
Casein (Difco 14.92% N)	281.5 g
$CaCl_2$	83.25 g

APPENDIX 6

Lodgepole pine growth and nitrogen uptake

1. Average Growth Curve for Lodgepole Pine. This is based on the published data of Hocking 1972, Endean 1971 and Etter 1969.



2. Hypothetical Lodgepole Pine Nitrogen Consumption

Age From Seeding	Avg Dry Wt (mg)	mg N/ plant	Avg Uptake/wk
0-6 wks	25	.63	.105
6-10 wks	65	1.63	.20
10-12 wks	150	3.75	1.06
12-18 wks	500	12.5	1.46
18-20 wks	650	16.25	1.88

Average values for dry weight are taken from the "Average Growth Curve". Nitrogen content per plant and resultant weekly demand is calculated by assuming an optimally growing seedling will contain 2.5% N.

3. Nitrogen Content for Various Volumes of Organic Nitrogen Feed Solution (Conc)

Volume of Concentrate	mg Nitrogen Inorganic	Organic	Total
1 ml	7.13	21.4	28.5
.9	6.41	19.24	25.65
.8	5.70	17.10	22.80
.7	4.99	14.96	19.95
.6	4.28	12.82	17.10
.5	3.56	10.69	14.25
.4	2.85	8.55	11.40
.3	2.14	6.14	8.55
.2	1.43	4.27	5.70
.1	.71	2.14	2.85

4. Nitrogen Availability for Organic Nitrogen Feeding Based on 10 Plants per Chamber[1]

Age From Sterilization[2]	Mls Conc Added	Nitrogen Added			Inorganic Consumed	Total N Remaining	Balance[3] of Inorganic N
		Organic	Inorganic	Total			
0-4 wks							
4 wks	.5	10.69	3.56	14.25	4.20	10.05	-0.64
5 wks	.1	2.14	.71	2.85	1.05	11.85	-0.98
6 wks	.1	2.14	.71	2.85	2.00	12.70	-2.27
7 wks	.1	2.14	.71	2.85	2.00	13.55	-3.56
8 wks	.1	2.14	.71	2.85	2.00	14.40	-4.85
9 wks	.1	2.14	.71	2.85	2.00	15.25	-6.14
Harvest 65 days							
10 wks	.1	2.14	.71	2.85	10.60	7.50	-16.03
11 wks	.3	6.41	2.41	8.55	10.60	5.45	-24.49
12 wks	.4	8.55	2.85	11.40	14.60	2.25	-36.24
13 wks	.6	12.82	4.28	17.10	14.60	4.75	-46.56
14 wks	.6	12.82	4.28	17.10	14.60	7.25	-56.88
Harvest 101 days							

1) Calculated from hypothetical growth curve assumed N values.

2) Age from sterilization is the same as age from seeding.

3) Balance at the end of the week noted.

APPENDIX 7

Organism Counts from Young and Mature Tree Sampling

Organism Counts From Four Sampled Young Trees

Sample	Total Counts per g	Standard Deviation	Total Counts per 100 sq cm	$^{R}/_{S}$ *	$^{R}/_{S}$ **
Control Soil					
# 1	14×10^4	$\pm 2.5 \times 10^4$	18.1		
# 2	15×10^4	$\pm 1.9 \times 10^4$	19.8		
# 3	51×10^4	$\pm 9.6 \times 10^4$	54.5		
# 4	39×10^4	$\pm 1.7 \times 10^4$	42.9		
Rhizosphere Soil					
# 1	42×10^5	$\pm 2.9 \times 10^5$	538	30	
# 2	89×10^5	$\pm 6.3 \times 10^5$	1206	61	
# 3	68×10^5	$\pm 3.9 \times 10^5$	718	13	
# 4	73×10^5	$\pm 8.2 \times 10^5$	815	19	
Rhizoplane					
# 1	52×10^6	$\pm 4.9 \times 10^6$	48×10^6	367	27×10^5
# 2	106×10^6	$\pm 9.3 \times 10^6$	97×10^6	726	49×10^5
# 3	56×10^6	$\pm 7.3 \times 10^6$	51×10^6	108	9×10^5
# 4	109×10^6	$\pm 12 \times 10^6$	100×10^6	282	23×10^5

* Comparison based on total counts/gram of material

** Comparison based on total counts/100 sq centimeters

Organism Counts From Mature Tree Sampling

	CONTROL			RHIZOSPHERE				RHIZOPLANE				
Sample No	Total Counts per gram	Surface Area per g	Total Counts per 100 sq cm	Total Counts per gram	Root Diameter mm	Total Counts per 100 sq cm		Total Counts per gram	Surface Area per gram	Total Counts per 100 sq cm	R/S*	R/S**
I TC_1	49×10^4			57×10^4								
II TC_1	73×10^4			112×10^4				147×10^4	23.1	637×10^4		
III TC_1	78×10^4							230×10^4	23.4	985×10^4		
AVE	67×10^4	$14.9m^2$	447	85×10^4	41	571	1.3	188×10^4		811×10^4	2.8	1.8×10^4
I TC_2	73×10^4			274×10^4								
II TC_2	93×10^4			78×10^4				552×10^4	31.5	1750×10^4		
III TC_2	191×10^4							745×10^4	27.6	2720×10^4		
AVE	119×10^4	$14.9m^2$	798	176×10^4	17	1180	1.5	648×10^4		2235×10^4	3.4	2.8×10^4
I TC_3	85×10^4			857×10^4								
II TC_3	62×10^4			321×10^4								
III TC_3	103×10^4							384×10^5	29.9	1280×10^5		
AVE	84×10^4	$14.9m^2$	561	589×10^4	2.52	3950	7.2	384×10^5		1280×10^5	4.6	2.3×10^5
I TC_4	30×10^4			185×10^4								
II TC_4	20×10^4			599×10^4								
III TC_4	52×10^4							121×10^5	31.4	387×10^5		
AVE	34×10^4	$14.9m^2$	225	392×10^4	2.26	2630	10.2	121×10^5		387×10^5	36	1.7×10^5
III TC_5	28×10^3	$14.9m^2$	18.7		1.44			202×10^5	43.3	466×10^5	722	2.5×10^5
I TR_2S		$14.9m^2$			1.60			772×10^5	36.9	2090×10^5	65	2.6×10^5
I TRM					1.08			286×10^5	50.2	572×10^5	24	7.2×10^4

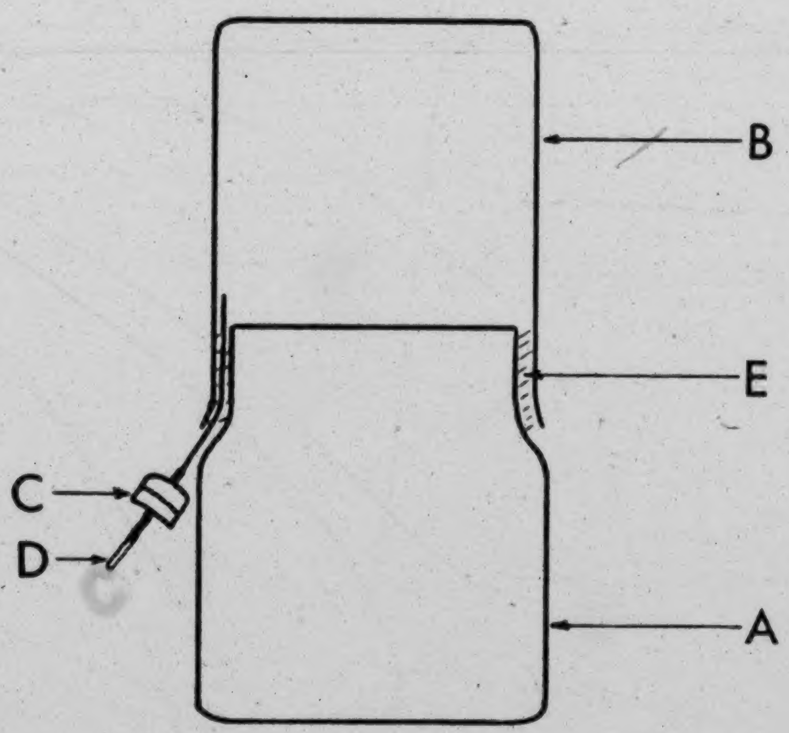
* Comparison based on total counts/gram of material

** Comparison based on total counts/100 sq centimeters

APPENDIX 8

Schematic Presentation of Sterile Growth Assemblies

1. System for Growing Sterile Plants with Pressurized Sterile Air Flow



A. One pint narrow mouth mason jar wrapped in foil and containing the soil mix.

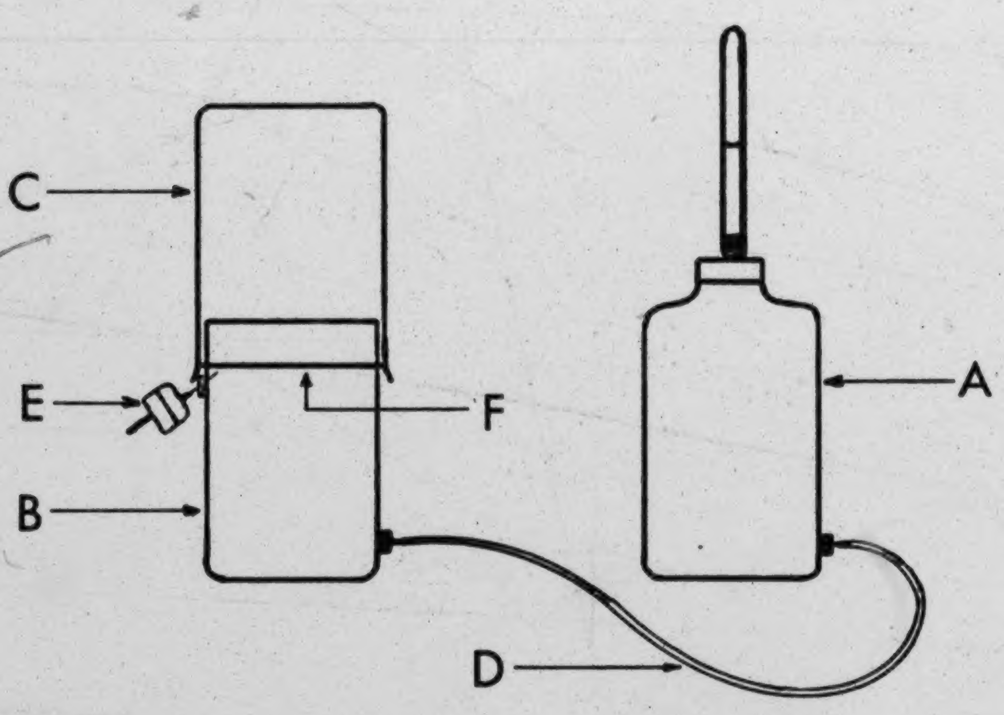
B. 600 ml beaker.

C. Filtered air entry system of 25 mm Swinnex filter, 16 G hypodermic needle and tygon tubing held in palce by an elastic band and tape.

D. 2.5 cm length of capillary tubing.

E. Cotton batting.

2. Sterile Plant Growth System with Pressurized Sterile Air Stream and Gravity Feed Nutrient Reservoir Attached.



A. 1 liter polypropylene bottle with nutrient solution and air filter.

B. 1 liter polypropylene bottle cut off just below neck.

C. 800 ml beaker

D. Nutrient exchange line of rubber tubing with entry to the polypropylene bottle via 16 G needles pushed through 16 mm serum stoppers.

E. Sterile air filtration system of 25 mm Swinnex filter held in palce by a 16 mm serum stopper.

F. Rubber "O" ring.

APPENDIX 9

Physiological capabilities of pure cultures used in micro-organism plant growth interaction study.

1) Organism Selection

The organisms 14b and 47c were selected from the rhizoplane isolates. As such they demonstrated a positive response to amiono acid supplementation of basal medium. These isolates when grown on this medium only produce a low optical density reading after 72 hr growth at 20°C and pH 6.8. This reaction is typical of rhizoplane isolates.

These two were chosen so the effect of supplying the plant with different nitrogen forms converted from organic nitrogen. The isolate 14b was proteolytic and isolate 47c was both proteolytic and an ammonifier. Other characteristics of these isolates are presented below.

2) Organism Characteristics

	Organism	
	47c	14b
Oxidase	+	-
Catalase	+	+
Nitrate Reduction	-	-
Acid from Glucose	-	+
Alkali from Lactose	-	+
Growth 37°C	+	+
Growth 3-4°C	-	-
Methylene Blue Reduction	+	+

		Organism	
		47c	14b
Urease		+	-
Chitinase		+	-
Lipase		-	+
Phosphatase		-	-
Amylase		-	-
PO_4-solubilization		-	-
Growth pH	4.6	-	+
	5.0	-	+
	5.6	+	+